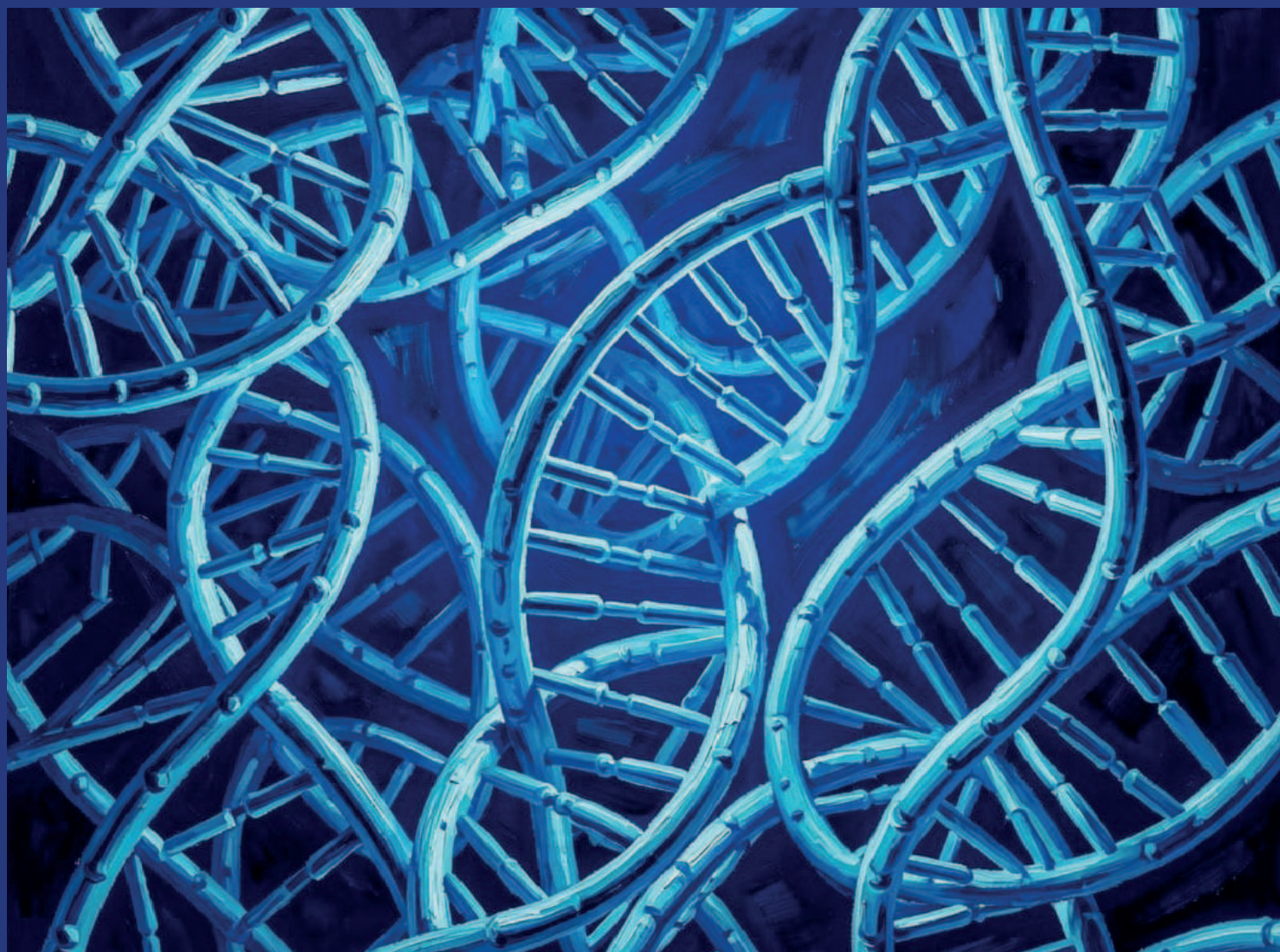


Vol. 28 No. 1 / 2026

ISSN Print 2713-2757
ISSN Online 2713-2765

EXTREME MEDICINE

Journal of FMBA of Russia



**MAIN TOPIC: GENOMIC AND POST GENOMIC
TECHNOLOGIES FOR THE DEVELOPMENT
OF PERSONALIZED MEDICINE**

EXTREMEMEDICINE.RU



EXTREME MEDICINE

Frequency of 4 issues per year.
Founded in 1999

SCIENTIFIC AND PRACTICAL JOURNAL OF FMBA OF RUSSIA

EDITOR-IN-CHIEF

Veronika Skvortsova,
DSc, professor, member of the RAS

DEPUTY EDITOR-IN-CHIEF

Igor Berzin, DSc, professor
Daria Kryuchko, DSc

EXECUTIVE EDITOR

Lilia Korsun, PhD

EDITORS

Olga Lalymenko, PhD
Alexander Biryuzov
Maria Poberezhnaya

SCIENTIFIC EDITORS

Vsevolod Belousov, DSc, professor, member of the RAS
Anton Keskinov, PhD

TRANSLATOR

Alexander Biryuzov

DESIGN

Maria Poberezhnaya

EDITORIAL BOARD

Bogomolov AV, DSc, professor (Moscow, Russia)
Boyko AN, DSc, professor (Moscow, Russia)
Bolekhan WN, DSc, docent (Moscow, Russia)
Borisevich IV, DSc, professor (Moscow, Russia)
Bushmanov AY, DSc, professor (Moscow, Russia)
Valenta R, PhD, professor (Vienna, Austria)
Voskanyan S, member of the RAS, DSc, professor (Moscow, Russia)
Daikhes NA, member of the RAS, DSc, professor (Moscow, Russia)
Dudarenko SV, DSc, professor (Saint-Petersburg, Russia)
Zykov KA, member of the RAS, DSc, professor (Moscow, Russia)
Karkischenko NN, member of the RAS, DSc, professor (Moscow, Russia)
Kaspranskiy RR, PhD (Moscow, Russia)
Lagarkova MA, member of the RAS, DSc, professor (Moscow, Russia)
Lobzin YV, member of the RAS, DSc, professor (Saint-Petersburg, Russia)
Nikiforov VV, DSc, professor (Moscow, Russia)

Olesova VN, DSc, professor (Moscow, Russia)
Petrov RV, member of the RAS, DSc, professor (Moscow, Russia)
Polyaev BA, DSc (Moscow, Russia)
Radilov AS, DSc, professor (Saint-Petersburg, Russia)
Rejniuk VL, DSc, docent (Saint-Petersburg, Russia)
Rembovsky VR, DSc, professor (Saint-Petersburg, Russia)
Sidorenko SV, member of the RAS, DSc, professor (Saint-Petersburg, Russia)
Sidorkevich SV, DSc (Moscow, Russia)
Styazhkin KK, DSc, professor (Moscow, Russia)
Troitsky AV, DSc, professor (Moscow, Russia)
Uskov AN, DSc, docent (Saint-Petersburg, Russia)
Ushakov IB, member of the RAS, DSc, professor (Moscow, Russia)
Khaitov MR, member of the RAS, DSc, professor (Moscow, Russia)
Yudin SM, DSc, professor (Moscow, Russia)

ADVISORY BOARD

Akleev AV, DSc, professor (Chelyabinsk, Russia)
Arakelov SA, PhD, professor (Saint-Petersburg, Russia)
Baklaushev VP, DSc, professor (Moscow, Russia)
Efimenko NV, DSc, professor (Pyatigorsk, Russia)
Kazakevich EV, DSc, professor (Arkhangelsk, Russia)
Katuntsev VP, DSc, professor (Moscow, Russia)
Klimanov VA, DSc, professor (Moscow, Russia)
Klinov DV, PhD (Moscow, Russia)
Minnullin IP, DSc, professor (Saint-Petersburg, Russia)
Mosyagin IG, DSc, professor (Saint-Petersburg, Russia)

Panasenko OM, DSc, member of the RAS, professor (Moscow, Russia)
Rogozhnikov VA, DSc, (Moscow, Russia)
Sotnichenko SA, DSc (Vladivostok, Russia)
Suranova TG, PhD, docent (Moscow, Russia)
Takhauov RM, DSc, professor (Seversk, Russia)
Shandala NK, DSc, professor (Moscow, Russia)
Shinkarev SM, DSc (Moscow, Russia)
Shipulin GA, PhD (Moscow, Russia)
Yakovleva TV, DSc (Moscow, Russia)

Founder

FMBA of Russia, Volokolamskoe shosse 30, bld. 1, Moscow 123182, Russia

Publisher

Centre for Strategic Planning of the Federal Medical and Biological Agency,
Pogodinskaya St. 1, bld. 10, Moscow 119121, Russia

Postal address of the editorial office

Pogodinskaya St. 1, bld. 10, Moscow 119121, Russia
extrememedicine@cspfmba.ru; www.extrememedicine.ru

Contract publisher

NEICON ISP LLC: Letnikovskaya St. 4/5, Moscow 115114, Russia

Printing office

Triada Publishing House LLC: Tchaikovsky Ave 9, office 514, Tver 170034, Russia

Print run

100 copies. Free price

Passed for printing

12 Mar. 2026

Date of publication

30 Mar. 2026

The journal is registered as a mass medium by the Federal Service for Supervision of Communications, Information Technologies and Mass Communications.

Certificate PI No. FS 77-25124 dated 27 July 2006
The content is licensed under the Creative Commons Attribution 4.0 International licence (CC BY 4.0).

Scopus®

Indexed in Scopus in 2022

**НАУЧНАЯ ЭЛЕКТРОННАЯ
БИБЛИОТЕКА
LIBRARY.RU**

Indexed in RSCI. IF 2023: 0,541



**ВЫСШАЯ
АТТЕСТАЦИОННАЯ
КОМИССИЯ (ВАК)**

Listed in HAC 08.10.2024 (№ 1668)

CYBERLENINKA

Open access to archive

© FMBA of Russia, 2026

© Centre for Strategic Planning of the Federal Medical and Biological Agency, 2026

МЕДИЦИНА ЭКСТРЕМАЛЬНЫХ СИТУАЦИЙ

НАУЧНО-ПРАКТИЧЕСКИЙ ЖУРНАЛ ФМБА РОССИИ

Периодичность 4 номера в год.
Основан в 1999 году

ГЛАВНЫЙ РЕДАКТОР

Вероника Скворцова,
д. м. н., профессор, член-корр. РАН

ЗАМЕСТИТЕЛИ ГЛАВНОГО РЕДАКТОРА

Игорь Берзин, д. м. н., профессор
Дарья Крючко, д. м. н., доцент

ОТВЕТСТВЕННЫЙ РЕДАКТОР

Лилия Корсун, к. б. н.

РЕДАКТОРЫ

Ольга Лалыменко, к. м. н.
Александр Бирюзов
Мария Побережная

НАУЧНЫЕ РЕДАКТОРЫ

Всеволод Белоусов, д. б. н., профессор, член-корр. РАН
Антон Кескинов, к. м. н.

ПЕРЕВОДЧИК

Александр Бирюзов

ДИЗАЙН

Мария Побережная

РЕДАКЦИОННАЯ КОЛЛЕГИЯ

А. В. Богомолов, д. т. н., профессор (Москва, Россия)
А. Н. Бойко, д. м. н., профессор (Москва, Россия)
В. Н. Болехан, д. м. н., доцент (Москва, Россия)
И. В. Борисевич, д. м. н., профессор (Москва, Россия)
А. Ю. Бушманов, д. м. н., профессор (Москва, Россия)
Р. Валента, PhD, профессор (Вена, Австрия)
С. Э. Восканян, д. м. н., профессор, член-корр. РАН (Москва, Россия)
Н. А. Дайхес, д. м. н., профессор, член-корр. РАН (Москва, Россия)
С. В. Дударенко, д. м. н., профессор (Санкт-Петербург, Россия)
К. А. Зыков, д. м. н., профессор РАН, член-корр. РАН (Москва, Россия)
Н. Н. Каркищенко, д. м. н., профессор, член-корр. РАН (Москва, Россия)
Р. Р. Каспранский, к. м. н. (Москва, Россия)
М. А. Лагарькова, д. б. н., профессор, член-корр. РАН (Москва, Россия)
Ю. В. Лобзин, д. м. н., профессор, академик РАН (Санкт-Петербург, Россия)
В. В. Никифоров, д. м. н., профессор (Москва, Россия)

В. Н. Олесова, д. м. н., профессор (Москва, Россия)
Р. В. Петров, д. м. н., профессор, академик РАН (Москва, Россия)
Б. А. Поляев, д. м. н., профессор (Москва, Россия)
А. С. Радилов, д. м. н., профессор (Санкт-Петербург, Россия)
В. Л. Рейнюк, д. м. н., доцент (Санкт-Петербург, Россия)
В. Р. Рембовский, д. м. н., профессор (Санкт-Петербург, Россия)
С. В. Сидоренко, д. м. н., профессор, член-корр. РАН (Санкт-Петербург, Россия)
С. В. Сидоркевич, д. м. н. (Москва, Россия)
К. К. Стяжкин, д. б. н., профессор (Москва, Россия)
А. В. Троицкий, д. м. н., профессор (Москва, Россия)
А. Н. Усков, д. м. н., доцент (Санкт-Петербург, Россия)
И. Б. Ушаков, д. м. н., профессор, академик РАН (Москва, Россия)
М. Р. Хаитов, д. м. н., профессор, член-корр. РАН (Москва, Россия)
С. М. Юдин, д. м. н., профессор (Москва, Россия)

РЕДАКЦИОННЫЙ СОВЕТ

А. В. Аклев, д. м. н., профессор (Челябинск, Россия)
С. А. Араkelов, к. б. н., профессор (Санкт-Петербург, Россия)
В. П. Баклашев, д. м. н., профессор (Москва, Россия)
Н. В. Ефименко, д. м. н., профессор (Пятигорск, Россия)
Е. В. Казакевич, д. м. н., профессор (Архангельск, Россия)
В. П. Катунцев, д. м. н., профессор (Москва, Россия)
В. А. Климанов, д. ф.-м. н., профессор (Москва, Россия)
Д. В. Клинов, к. ф.-м. н. (Москва, Россия)
И. П. Миннуллин, д. м. н., профессор (Санкт-Петербург, Россия)
И. Г. Мосягин, д. м. н., профессор (Санкт-Петербург, Россия)

О. М. Панасенко, д. б. н., профессор, член-корр. РАН (Москва, Россия)
В. А. Рогожников, д. м. н., профессор (Москва, Россия)
С. А. Сотниченко, д. м. н. (Владивосток, Россия)
Т. Г. Суранова, к. м. н., доцент (Москва, Россия)
Р. М. Тахауов, д. м. н., профессор (Северск, Россия)
Н. К. Шандала, д. м. н., профессор (Москва, Россия)
С. М. Шинкарев, д. т. н. (Москва, Россия)
Г. А. Шипулин, к. м. н. (Москва, Россия)
Т. В. Яковлева, д. м. н. (Москва, Россия)

Учредитель

ФМБА России, 123182, Москва, Волоколамское шоссе, д. 30, стр. 1

Издатель

ФГБУ «ЦСП» ФМБА России, 119121, Москва, Погодинская ул., д. 10, стр. 1

Адрес редакции

119121, Москва, Погодинская ул., д. 10, стр. 1
extrememedicine@cspsfmba.ru; www.extrememedicine.ru

Исполнитель

ООО «НЭИКОН ИСП»: 115114, Москва, Летниковская ул., д. 4, стр. 5

Типография

ООО «Издательство «Триада»: 170034, Тверь, Чайковского пр., д. 9, оф. 514

Тираж

100 экз. Цена свободная

Подписано в печать

12.03.2026

Дата выхода в свет

30.03.2026

Журнал зарегистрирован в Федеральной службе по надзору в сфере связи, информационных технологий и массовых коммуникаций.

Свидетельство ПИ № ФС77-25124 от 27 июля 2006 г.
Контент доступен по лицензии Creative Commons Attribution International CC BY 4.0.

Scopus®

Журнал включен в Scopus в 2022 г.

НАУЧНАЯ ЭЛЕКТРОННАЯ
БИБЛИОТЕКА
LIBRARY.RU

Журнал включен в РИНЦ. IF 2023: 0,541



ВЫСШАЯ
АТТЕСТАЦИОННАЯ
КОМИССИЯ (ВАК)

Журнал включен в Перечень 08.10.2024 (№ 1668)

CYBERLENINKA

Здесь находится открытый архив

MAIN TOPIC:

GENOMIC AND POST-GENOMIC TECHNOLOGIES
AS A BASIS FOR THE DEVELOPMENT
OF PERSONALIZED MEDICINE

Issues of legislative regulation in the field of human genetic data

D.A. Kashtanova, A.A. Mamchur, A.Yu. Yakovchik, A.R. Grigoryan,
M.V. Ivanov, S.I. Mitrofanov, I.N. Svidovskaya, V.S. Yudin, A.A. Keskinov,
S.M. Yudin

Hidden burden of chronic non-communicable diseases.
Part 1: Prevalence of germline variants associated with
cardiovascular diseases in Russian population

V.S. Yudin, A.A. Mamchur, M.V. Ivanov, E.A. Nikolaeva, D.A. Kashtanova,
V.V. Daniel, S.I. Mitrofanov, A.A. Keskinov, L.P. Kuzmina, S.M. Yudin

Hidden burden of chronic non-communicable diseases.
Part 2: Prevalence of germline variants associated with
cancers among residents of the Russian Federation

V.S. Yudin, A.A. Mamchur, E.A. Nikolaeva, M.V. Ivanov, D.A. Kashtanova,
E.A. Zelenova, S.I. Mitrofanov, E.A. Snigir, L.P. Kuzmina, A.A. Keskinov

Allelic variants of CYP2C9, CYP2C19, CYP2D6 cytochromes
in the Russian population: Prevalence and regional
distribution

V.S. Yudin, E.D. Spektor, A.A. Mamchur, M.V. Ivanov, S.I. Mitrofanov,
L.P. Kuzmina, I.V. Bukhtiyarov, A.A. Keskinov, S.M. Yudin, D.A. Kashtanova

Pharmacogenetic factors determining the metabolism
and safety of aromatic anticonvulsants in the residents
of Russia

E.D. Spektor, V.S. Yudin, A.A. Mamchur, A.M. Rumyantseva, M.V. Ivanov,
S.I. Mitrofanov, E.A. Snigir, A.A. Keskinov, S.M. Yudin, D.A. Kashtanova

CELL BIOLOGY

Optimization of initial culture stage of Vero and HEK293
cell lines

I.I. Tuzova, T.I. Chirkina, I.A. Churkin, A.N. Lyakh, K.M. Mefed,
V.A. Maximov

RADIOBIOLOGY

Evaluation of the frequency of ultra-short and ultra-long
telomeres in chronically exposed women

Ya.V. Krivoshchapova, Yu.R. Akhmadullina

SPORTS MEDICINE

Significance of QT interval prolongation in youth and
adolescent sports (literature review)

L.M. Makarov, V.N. Komoliatova, A.G. Akopyan, D.A. Besportochnyi,
A.V. Dmitrieva, I.I. Kiseleva, I.V. Zybkin

Effect of rhythmic peripheral magnetic stimulation
on the functional capabilities of the musculoskeletal system
of male athletes

Y.V. Koryagina, J.V. Kushnareva, S.V. Nopin, S.M. Abutailimova, I.N. Mitin

Assessment of the relationship between tracheal breathing
sounds and lung ventilation during physical exercise

S.N. Astafyeva, A.I. Dyachenko, I.A. Ruzhichko, A.E. Kostiv

ГЛАВНАЯ ТЕМА:

ГЕНОМНЫЕ И ПОСТГЕНОМНЫЕ
ТЕХНОЛОГИИ КАК ОСНОВА РАЗВИТИЯ
ПЕРСОНАЛИЗИРОВАННОЙ МЕДИЦИНЫ

5

Проблемы законодательного регулирования в области
обращения генетических данных человека

Д.А. Каштанова, А.А. Мамчур, А.Ю. Яковчик, А.Р. Григорян,
М.В. Иванов, С.И. Митрофанов, И.Н. Свидовская, В.С. Юдин,
А.А. Кескинов, С.М. Юдин

19

Скрытое бремя хронических неинфекционных
заболеваний. Часть 1: распространенность
герминальных вариантов, ассоциированных с сердечно-
сосудистыми заболеваниями, среди населения
Российской Федерации

В.С. Юдин, А.А. Мамчур, М.В. Иванов, Е.А. Николаева,
Д.А. Каштанова, В.В. Даниэль, С.И. Митрофанов, А.А. Кескинов,
Л.П. Кузьмина, С.М. Юдин

28

Скрытое бремя хронических неинфекционных
заболеваний. Часть 2: распространенность герминальных
вариантов, ассоциированных с онкологическими
заболеваниями, среди жителей Российской Федерации

В.С. Юдин, А.А. Мамчур, Е.А. Николаева, М.В. Иванов,
Д.А. Каштанова, Е.А. Зеленова, С.И. Митрофанов, Е.А. Снигирь,
Л.П. Кузьмина, А.А. Кескинов

37

Аллельные варианты цитохромов CYP2C9, CYP2C19,
CYP2D6 у жителей России: распространенность
и региональное распределение

В.С. Юдин, Е.Д. Спектор, А.А. Мамчур, М.В. Иванов,
С.И. Митрофанов, Л.П. Кузьмина, И.В. Бухтияров, А.А. Кескинов,
С.М. Юдин, Д.А. Каштанова

51

Фармакогенетические факторы, определяющие
метаболизм и безопасность антиконвульсантов
с ароматической структурой у жителей России

Е.Д. Спектор, В.С. Юдин, А.А. Мамчур, А.М. Румянцев, М.В. Иванов,
С.И. Митрофанов, Е.А. Снигирь, А.А. Кескинов, С.М. Юдин,
Д.А. Каштанова

КЛЕТОЧНАЯ БИОЛОГИЯ

61

Оптимизация начального этапа культивирования
клеточных линий Vero и HEK293

И.И. Тузова, Т.И. Чиркина, И.А. Чуркин, А.Н. Лях, К.М. Мефёд,
В.А. Максимов

РАДИОБИОЛОГИЯ

69

Оценка частоты сверхкоротких и сверхдлинных теломер
у хронически облученных женщин

Я.В. Кривошапова, Ю.Р. Ахмадуллина

СПОРТИВНАЯ МЕДИЦИНА

79

Значение удлинения интервала QT в детско-юношеском
спорте (обзор литературы)

Л.М. Макаров, В.Н. Комолятова, А.Г. Акопян, Д.А. Беспорточный,
А.В. Дмитриева, И.И. Киселева, И.В. Зябкин

88

Влияние применения ритмической периферической
магнитной стимуляции на функциональные возможности
опорно-двигательного аппарата спортсменов-мужчин

Ю.В. Корягина, Ю.В. Кушнарева, С.В. Нопин, С.М. Абуталимова,
И.Н. Митин

95

Оценка зависимости мощности трахеальных
дыхательных шумов от легочной вентиляции
при физической нагрузке

С.Н. Астафьева, А.И. Дьяченко, И.А. Ружичко, А.Е. Костив

SPACE MEDICINE

Epidemiological analysis of cosmonaut mortality: 1960–2023
I.B. Ushakov, A.Yu. Bushmanov, K.V. Betts, I.V. Bukhtiyarov, Yu.I. Voronkov,
A.P. Grishin, A.F. Zhernavkov

ARCTIC MEDICINE

Requirements for medical personnel providing medical care at onsite industrial facilities in the Russian Arctic continental shelf
O.I. Orlov, E.Yu. Mamonova, M.A. Romanov, M.Yu. Kalinina

EMERGENCY RESPONSE ORGANIZATION

Multistage surgical management of patients with combined craniofacial injuries
Yu.A. Shcherbuk, O.D. Madai, D.Yu. Madai, A.Yu. Shcherbuk

MICROBIOLOGY

Optimization and validation of *Bacillus subtilis* spore inactivation regimes in supercritical carbon dioxide: Pure gas, hydrogen peroxide, peracetic acid
R.Sh. Gvetadze, M.S. Galstyan, Ya.N. Kharakh, P.Yu. Kolesnikov,
L.G. Kirakosyan, M.S. Podporin, V.N. Tsarev, S.D. Arutyunov

CLINICAL PHARMACOLOGY

Honey-based hydrogel: A promising new-generation antimicrobial coating?
E.N. Karpina, E.V. Fedotova, F.F. Zaripova, D.V. Krivorotov, A.S. Radilov

КОСМИЧЕСКАЯ МЕДИЦИНА

102 **Эпидемиологический анализ смертности космонавтов: 1960–2023**
И.Б. Ушаков, А.Ю. Бушманов, К.В. Бетц, И.В. Бухтияров,
Ю.И. Воронков, А.П. Гришин, А.Ф. Жерनावков

АРКТИЧЕСКАЯ МЕДИЦИНА

113 **Требования к медицинскому персоналу, осуществляющему медицинскую помощь на стационарных производственных объектах в районе Арктического шельфа России**
О.И. Орлов, Е.Ю. Мамонова, М.А. Романов, М.Ю. Калинина

ОРГАНИЗАЦИЯ ПОМОЩИ В ЧРЕЗВЫЧАЙНЫХ СИТУАЦИЯХ

123 **Многостадийное хирургическое лечение пострадавших с сочетанными черепно-лицевыми повреждениями головы**
Ю.А. Щербук, О.Д. Мадай, Д.Ю. Мадай, А.Ю. Щербук

МИКРОБИОЛОГИЯ

134 **Оптимизация и валидация режимов инактивации спор *Bacillus subtilis* в среде сверхкритического CO₂: чистый газ, перекись водорода, надуксусная кислота**
Р.Ш. Гветадзе, М.С. Галстян, Я.Н. Харах, П.Ю. Колесников,
Л.Г. Киракосян, М.С. Подпорин, В.Н. Царев, С.Д. Арутюнов

КЛИНИЧЕСКАЯ ФАРМАКОЛОГИЯ

146 **Медовый гидрогель — перспективное антибактериальное покрытие нового поколения?**
Е.Н. Карпина, Е.В. Федотова, Ф.Ф. Зарипова, Д.В. Криворотов,
А.С. Радилов

<https://doi.org/10.47183/mes.2025-330>



ISSUES OF LEGISLATIVE REGULATION IN THE FIELD OF HUMAN GENETIC DATA

Daria A. Kashtanova[✉], Aleksandra A. Mamchur, Anna Yu. Yakovchik, Aram R. Grigoryan, Mikhail V. Ivanov, Sergey I. Mitrofanov, Irina N. Svidovskaya, Vladimir S. Yudin, Anton A. Keskinov, Sergey M. Yudin

Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical and Biological Agency, Moscow, Russia

Introduction. Genetic data, which are subject to legal protection, require the creation of specialized legislative acts regulating the collection, processing, and storage of such information. The development of a conceptual framework that could take into account the diversity of genetic data types and their application purposes represents a relevant task.

Objective. Determining directions for the development of a regulatory framework governing the circulation of human genetic data in the Russian Federation based on an assessment of the current state of legal regulation in this field.

Discussion. All types of genetic information are subject to legal protection according to their significance. In terms of current terminology, such concepts as “genetic information” and “genetic data,” require clarification. The Russian legislation is currently lacking a regulatory document governing the circulation of genetic data and genetic information obtained from humans. Foreign experience demonstrates active adoption of specialized legal acts protecting genetic information. Active development of biobanks and databases of genetic information requires clear legislative algorithms for data protection. In cases of anonymized data stored in databases, personal or medical data protection regimes become impractical. Thus, databases should occupy a separate position in the legal landscape. Issues regarding the regulation of creation, storage, and dissemination of genetic data repositories remain unresolved. Approaches to protecting and securing individual-level genetic information (in the context of medical care or private use) and safeguarding anonymous genetic data banks (for research purposes) should be delimited.

Conclusions. The existing disparity between the current advances in genetic technologies and legislation governing the generated data can be resolved by introducing new legislative initiatives and enacting laws that take into account the knowledge of modern biomedical science.

Keywords: genetic technologies; legislative and legal framework; biobank; genetic data; database of population frequencies of genetic variants; legal protection

For citation: Kashtanova D.A., Mamchur A.A., Yakovchik A.Yu., Grigoryan A.R., Ivanov M.V., Mitrofanov S.I., Svidovskaya I.N., Yudin V.S., Keskinov A.A., Yudin S.M. Issues of legislative regulation in the field of human genetic data. *Extreme Medicine*. 2026;28(1):5–18. <https://doi.org/10.47183/mes.2025-330>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: Sergey M. Yudin is an Editorial Board member and Anton A. Keskinov is the scientific editor of *Extreme Medicine*. The other authors declare no conflict of interest.

✉ Daria A. Kashtanova DKashtanova@cspfmbsa.ru

Received: 19 June 2025 **Revised:** 5 Sep. 2025 **Accepted:** 1 Oct. 2025 **Online first:** 24 Oct. 2025

УДК 575.113.1

ПРОБЛЕМЫ ЗАКОНОДАТЕЛЬНОГО РЕГУЛИРОВАНИЯ В ОБЛАСТИ ОБРАЩЕНИЯ ГЕНЕТИЧЕСКИХ ДАННЫХ ЧЕЛОВЕКА

Д.А. Каштанова[✉], А.А. Мамчур, А.Ю. Яковчик, А.Р. Григорян, М.В. Иванов, С.И. Митрофанов, И.Н. Свидовская, В.С. Юдин, А.А. Кескинов, С.М. Юдин

Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

Введение. Генетические данные, являющиеся объектом правовой защиты, требуют создания специализированных законодательных актов, регулирующих сбор, обработку и хранение подобного рода информации. Разработка категориально-понятийного аппарата с учетом многообразия как видов генетической данных, так и целей, для которых они могут быть использованы, является актуальной задачей.

Цель. Определение направлений развития нормативно-правовой базы, регулирующей обращение с генетическими данными человека в Российской Федерации, на основе оценки текущего состояния правового регулирования данной сферы.

Обсуждение. Правовой охране должны подлежать все возможные виды генетической информации в соответствии с их значимостью. В используемом понятийном аппарате существуют слабые места при определении понятий «генетическая информация» и «генетические данные», которые нужно учесть в дальнейшей работе в этом направлении. В настоящее время на территории Российской Федерации отсутствует какой-либо нормативный документ, регулирующий обращение генетических данных и генетической информации, полученных от людей. Зарубежный опыт правового регулирования обращения с генетической информацией демонстрирует активную разработку и принятие специализированных нормативно-правовых актов в сфере защиты генетической информации. Активное создание биобанков и баз данных генетической информации влечет за собой создание четкого законодательного алгоритма защиты. При обезличенности данных, собранных в базы, применение режима защиты, предназначенного персональным или медицинским данным, невозможно. Таким образом, базы данных должны занимать особое положение в правовом поле. Остается открытым вопрос о регулировании создания, хранения и распространения баз генетических данных.

© D.A. Kashtanova, A.A. Mamchur, A.Yu. Yakovchik, A.R. Grigoryan, M.V. Ivanov, S.I. Mitrofanov, I.N. Svidovskaya, V.S. Yudin, A.A. Keskinov, S.M. Yudin, 2025

Обнаруживается необходимость разграничить подходы к обеспечению защиты и конфиденциальности генетической информации конкретного человека в рамках оказания медицинской помощи или при личном использовании и защиты баз генетической информации обезличенных индивидов в научно-исследовательских целях.

Выводы. Существующий диссонанс между прогрессом в области генетических технологий и законодательством в сфере регулирования получаемых данных предлагается решить выдвижением новых законодательных инициатив и принятием законодательных актов, учитывающих знания современной биомедицинской науки.

Ключевые слова: генетические технологии; законодательно-правовая база; биобанк; генетические данные; база данных популяционных частот генетических вариантов; правовая защита

Для цитирования: Каштанова Д.А., Мамчур А.А., Яковчик А.Ю., Григорян А.Р., Иванов М.В., Митрофанов С.И., Свидовская И.Н., Юдин В.С., Кескинов А.А., Юдин С.М. Проблемы законодательного регулирования в области обращения генетических данных человека. *Медицина экстремальных ситуаций*. 2026;28(1):5–18. <https://doi.org/10.47183/mes.2025-330>

Финансирование: исследование выполнено без спонсорской поддержки.

Потенциальный конфликт интересов: С.М. Юдин — член редакционной коллегии журнала «Медицина экстремальных ситуаций», А.А. Кескинов — научный редактор журнала «Медицина экстремальных ситуаций», остальные авторы заявляют об отсутствии конфликта интересов.

✉ Каштанова Дарья Андреевна DKashtanova@cspfmba.ru

Статья поступила: 19.06.2024 **После доработки:** 05.09.2025 **Принята к публикации:** 01.10.2025 **Online first:** 24.10.2025

INTRODUCTION

The widespread adoption of genetic research leads to the accumulation of a large volume of medical and personal data, the storage of which poses multiple ethical and legal risks. The current Russian legislative acts do not take into account specific features of this kind of information and, therefore, cannot provide for an adequate level of protection of such sensitive data. The existing discrepancy between progress in the field of genetic technologies and legislation regulating the data obtained may result in numerous legal disputes in the nearest future. Therefore, an immediate but balanced solution to the problem is necessary. This article discusses issues related to modern legislation in the field of regulating genetic data circulation, as well as probable challenges that may arise in the nearest future.

The key findings of the study:

- in Russia, a significant discrepancy between advancements in genetic technologies and legislation regulating the resulting data continue to exist;
- one of the most pressing issues is the lack of a unified terminology when defining the object of genetic data protection;
- the growing number of genetic studies and databases highlights the urgency of adequate regulation in this field;
- multidisciplinary teams consisting of lawyers, physicians, genetics scientists, and biotechnology experts should be formed to develop comprehensive legislative acts based on modern scientific knowledge.

Genetics (and clinical applications thereof) is one of the fastest-developing fields in biomedical science. Accessibility of laboratory and bioinformatic methods allowing decoding and interpretation of nucleic acid sequences leads to exponential growth in the volume of genetic information.¹ Its use spans widely, from diagnosing hereditary diseases to forensic medicine for identifying individuals.² Naturally, such data demands special protection and proper storage, since the human genome carries information about disease predispositions, ethnic affiliation, and other individual traits of both the person and their relatives [1]. Opportunities created by advanced technologies, big data, and applied scientific research pose fundamentally new challenges, giving rise to unprecedented risks that lie both in the medical and legal domains.

It should be noted that the rapid advancement of technological capabilities is accompanied by the growing demand for genetic tests, including commercial ones. According to media reports, in 2021 compared to 2020, companies in Russia specializing in genetic testing experienced a sales increase from 15% to 250%.³ According to the report by the Innovation Agency of Moscow published in September 2023, the market for genetic testing services has been expanding annually since 2021. In 2023, the estimated market size in Russia reached approximately 192.3 million US dollars. From 2021 to 2022, the market witnessed a significant surge, increasing by 16% from 2.5 billion rubles to 2.9 billion rubles. Over the next five years, the market is forecasted to grow annually by an average of 15.3%, having potentially reached 5 billion rubles by 2025.

¹ Bochkov NP, Puzyrev VP, Smirnikhina SA. Clinical genetics: textbook edited by Bochkov NP, 4th ed., revised and expanded; 2011.

² International Declaration on Human Genetic Data dated 16.10.2003. URL: https://www.un.org/ru/documents/decl_conv/declarations/genome_dec.shtml

³ Skobelev V. Losing weight and extending youth: why demand for genetic tests has increased in Russia. *Forbes Russia*. 2022. URL: <https://www.forbes.ru/tekhnologii/454171-sbrosit-ves-i-prodlit-molodost-pocemu-v-rossii-vyros-spros-na-geneticheskie-testy>

These trends underscore the rising demand for genetic testing services in Russia, driven by technological advancements and consumer awareness of personalized healthcare solutions.⁴ Following the completion of the COVID-19 pandemic in 2022–2023, the popularity of this service declined somewhat; however, many consulting companies express optimistic views on the development of the genetic testing market. According to some sources, it may grow from approximately 19.6 billion US dollars in 2023 to 65.03 billion US dollars in 2034, with the greatest growth occurring in the Asia-Pacific region⁵.

Unfortunately, the adequacy of protection for genetic data received by clinics and laboratories remains open to question. The situation that arose in October 2023 with one of the world's largest operators of genetic data — the American company 23andMe — when the data of nearly seven million users was stolen from the company's servers and subsequently sold on the black market,⁶ highlights the acute problem of securing information about human genetic characteristics. Apart from reputational losses, the company did not bear any real legal liability, demonstrating the imperfections of the current legislative regulation.

Obviously, the sensitivity of genetic data as an object of legal protection requires the creation of specialized legislative acts regulating the collection, processing, and storage of such information [2]. At present, however, the development of science, as a whole, and genetics, in particular, is outpacing updates to the corresponding legal framework. Legal acts regulating the rules for storing information in medical institutions (including physician–patient privilege) and laws on the protection of personal data are partly applicable to genetic data; however, as practice shows, they are unable to fully ensure an adequate level of security [3].

In the context of creation of a reliable legal framework for regulating work with genetic data and ensuring their security, the development of a categorical and conceptual framework is of particular importance. Such a framework should consider the variety of genetic data types and their application purposes. Given that this task cannot be solved without understanding deep biological fundamentals, it becomes obvious that close collaboration between legislators and specialists from various fields of knowledge, such as biomedicine, bioinformatics, and biotechnology, is necessary.

All across the globe, specialized bills aimed at regulating various aspects of work with genetic information (collection, processing, storage), and most importantly, ensuring data protection, are being developed and

adopted. At the same time, recent emerging challenges have led to a reassessment and updating of both general perspectives on the safety of genetic data and corresponding legal frameworks.

MATERIALS AND METHODS

The search for scientific literature was conducted in electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Google Scholar) languages. Normative documents were retrieved from the ConsultantPlus legal reference system (Russia) and using official websites of governments of different countries. The article reviews the situation in the legislative field of Russia and abroad regarding genetic information protection at the beginning of 2025.

The eLibrary, CyberLeninka, and Google Scholar databases were searched using the following keyword phases: “legal regulation of genetic data” and “laws in genetics.” In terms of depth, the search included scientific papers published from 2010 up to the present. The analysis also included publications and legislative acts describing legal relations in the field of circulation of genetic data obtained from humans.

Using the query “methods of genetic testing,” scientific publications dating back to 1990 until the present were retrieved. Publications describing methods used in today's genetic testing were included in the analysis.

RESULTS AND DISCUSSION

Types of genetic information subject to legal regulation

When developing regulations governing the handling of genetic data, it is essential to determine exactly which types of data require protection. Our research revealed that this question remains a key point of disagreement between the current legislative acts and genetics as a science.

From a biological perspective, the molecule of deoxyribonucleic acid (DNA) contained in the nucleus (nuclear DNA) and mitochondria (mitochondrial DNA) of a human cell serves as the primary carrier of inherited, i.e., genetic, information. DNA consists of numerous constituent elements referred to as nucleotides, the sequence of which encodes information. Collectively, all DNA molecules in a cell (approximately three billion nucleotides) constitute the genome. Each person's DNA sequence is unique (except for identical twins) and is determined

⁴ Technologies of genetic testing: global market focus on MENA Region and specific features of development in Russia. URL: https://psv4.userapi.com/s/v1/d/2tXlDm1ruzAtZMgZpzbiAWHnK_ekYfiI_loLGEJ6SAoWnvGgiCDK-mxen84-uwTHDC8sB57XWYVTKU7f3aUQiG82BdIA0dNQm_Vgi9cYAuZnND3Gq/AIM-Tekhnologii_geneticheskogo_testirovania_2023.pdf

⁵ Genetic Testing Market Size, Share, and Trends 2025 to 2034. URL: <https://www.precedenceresearch.com/genetic-testing-market>

⁶ Genetic testing firm 23andMe admits hackers accessed DNA data of 7m users. *The Guardian*. 2023. URL: <https://www.theguardian.com/technology/2023/dec/05/23andme-hack-data-breach>

at conception. Throughout one's lifetime, mutations may occur in DNA, but only at the level of individual cells, not the entire organism. Hence, DNA sequencing can serve as a tool for identifying individuals. Simultaneously, certain genetic variants are associated with the development of specific hereditary diseases, making DNA sequences clinically valuable. Nuclear DNA exists not as a single molecule but as 46 distinct chromosomes; deviations from this standard number represent clinically significant information, as they are linked to certain syndromes (such as Down syndrome).

However, DNA is not the sole carrier of genetic data. The realization of information encoded in DNA occurs through sequential reading of nucleotides and synthesis of ribonucleic acid (RNA) during transcription. The collective term for all RNA molecules in a cell is the transcriptome. Transcription occurs not from the entire DNA molecule but from specific segments known as genes. Different levels of gene activity result in varied copy numbers of individual RNAs: transcription of some genes may intensify when needed, while others diminish. Moreover, the activity of information readout from different DNA regions can additionally be regulated by epigenetic modifications within the DNA molecule itself (e.g., DNA methylation) and surrounding molecules (such as histone protein acetylation, which maintains DNA in a compact state). The sum of such modifications, in the case of DNA methylation, is referred to as the methylome. Both the transcriptome and methylome, alongside other epigenetic markers, can change over time and reflect an individual's current state, biological age, and environmental influences, making them vital clinical indicators. Some of these data can also be used for identifying individuals, albeit with a greater complexity compared to DNA analysis.

Thus, biologists define genetic information as encompassing details about the sequence of nuclear and mitochondrial DNA, the transcriptome, the proteome (the complete set of proteins produced by an organism), the methylome, and other epigenetic modifications.⁷ In its guidelines, the World Health Organization (WHO) also emphasizes that genetic data encompasses everything listed above and that this list is not complete,⁸ since the advancement of technologies for obtaining and analyzing genetic data may lead to its expansion and enrichment.

It is important to recognize that the analysis of genetic information can involve highly different degrees of data detail: technical capabilities of laboratories allow for both determining nucleotide sequences (of DNA or RNA) and simply counting the total number of chromosomes. Each method differs in its approach to sample

preparation, equipment, consumables, data analysis techniques, etc.

One of the simplest and quickest methods for detecting the presence of a target nucleotide sequence in the human genome is polymerase chain reaction (PCR). This method relies on repeatedly amplifying copies of a specific DNA fragment, usually 100–1000 nucleotides in length. The resulting fragments are visualized using gel electrophoresis to determine the length of the fragment [4]. When it is necessary to quantify the number of copies of the target DNA fragment, a variation referred to as real-time (quantitative) PCR is used. While PCR alone does not reveal nucleotide sequences, it can be applied for identifying individuals (e.g., paternity testing and forensics) and measuring the abundance of specific RNA transcripts [5].

In order to accurately determine or “read” the sequence of DNA or RNA, various sequencing methods are used. Among the most common are Sanger sequencing, NGS (Next Generation Sequencing), and nanopore sequencing. In Sanger sequencing, the nucleotide sequence of a strictly predefined target DNA fragment, typically 500–1000 nucleotides long, is determined [6]. NGS refers to a group of methods capable of simultaneously determining the sequences of many (up to billions) random short DNA fragments, each 50–300 nucleotides in length. Depending on sample preparation and the purpose of analysis, NGS can provide information about sequences of varying lengths (even complete genomes), although requiring preliminary fragmentation [7]. Nanopore sequencing enables the determination of both short and long nucleotide sequences. Its distinct feature is the ability to analyze multiple long sequences without prior genome fragmentation, enabling individual sequences to reach up to 100,000 nucleotides in length [8].

DNA microarrays fall between PCR and NGS methods in terms of utility. Similar to PCR, when using DNA microarrays, exact nucleotide sequences should be preselected in the sample. However, the scale of positions detected simultaneously can sometimes rival NGS and act as a substitute for this technology. With DNA microarrays, it is possible to identify tens to hundreds of thousands of individual positions in the genome and determine whether specific short sequences (~100 nucleotides) are present in the sample [9].

Light microscopy is the simplest, fastest, and most affordable method for assessing the number, size, and shape of chromosomes (without extracting DNA sequence information). To that end, chromosomes are isolated from cells at a specific lifecycle phase, stained, and examined under a microscope.

⁷ Guidance for human genome data collection, access, use and sharing; 2024. URL: <https://www.who.int/publications/i/item/9789240102149>

⁸ Ibid.

Table 1. Most common types of genetic information, acquisition methods, and data application domains

Acquired information about genetic data	Methods for acquiring genetic information	Applications of data (examples)
Individual positions in the genome or short DNA fragments	• DNA microarrays • PCR • Sanger sequencing • NGS	• clinical genetic tests and panels for assessing the risk of developing genetic disorders; • clinical testing to determine optimal therapy scenarios (pharmacogenetics)
Short Tandem Repeats (STRs), possessing a high mutation rate relative to other genomic regions, differing between individuals, and most similar among relatives	• PCR • Sanger sequencing • NGS	• state Genomic Registry; • paternity and kinship identification; • origin determination
Exome — the collection of DNA regions encoding protein structure information	• DNA microarrays • NGS • nanopore sequencing Sequencing by any other method that can yield information about the exome (WES)	• clinical genetic tests and panels for determining the risk of developing genetic diseases; • personal identification; • kinship testing; • origin determination; • creation of a human genetic passport (containing all the above-mentioned data)
Genome — the complete set of all hereditary information in an organism (totality of all DNA molecules in a cell)	• NGS • nanopore sequencing Sequencing by any other method that can yield information about the genome (WGS)	• clinical genetic tests and panels for assessing the risk of developing genetic disorders; • personal identification; • kinship testing; • origin determination; • creation of a human genetic passport (containing all the above-mentioned data); • research aimed at discovering genetic risk factors for diseases
Transcriptome — the sequence and copy number of RNA molecules in a cell	• DNA microarrays • quantitative (real-time) PCR • NGS • nanopore sequencing	• clinical tests for analyzing the expression levels of specific genes; • research focused on identifying molecular risk factors for diseases; • determination of biological age
Methylome: the methylation level of specific DNA regions	• DNA microarrays • NGS • nanopore sequencing	• determination of biological age; • clinical testing for the presence of specific methylation marks (in oncology and other fields); • research aimed at identifying epigenetic risk factors for diseases
Chromosome structure and number	• Light microscopy (for karyotyping) • NGS	• Clinical testing for chromosomal abnormalities (often performed during pregnancy planning and prenatal screening to detect genetic disorders in the fetus)

Table compiled by the authors

Note: DNA — Deoxyribonucleic Acid; PCR — Polymerase Chain Reaction; NGS — Next Generation Sequencing; WES — Whole Exome Sequencing; WGS — Whole Genome Sequencing.

Table 1 summarizes the main ways of utilizing the information obtained through the aforementioned methods.

Thus, genetic information encompasses a broad spectrum of data obtained through various methods, and all possible types of genetic information should be protected legally in accordance with their significance and sensitivity. Clearly, lawmaking should adopt such definitions of “genetic information” and “genetic data” that would align with those established in the scientific community. However, current legislative acts display significant flaws in their terminological framework, leading to the corresponding legal implications.

Federal Law No. 242-FZ⁹ employs the term “genomic information,” referring to “personal data comprising coded information about specific fragments of deoxyribonucleic acid belonging to a physical person or an unidentified corpse.” In this context, it is important to note certain discrepancies in the use of this term. As mentioned earlier, the concept of “genome” implies information about the complete DNA sequence in a cell, whereas under the framework of state genomic registration, only short tandem repeats (STR, Table 1) are considered. This allows for the identification of an individual or their relatives but does not provide information about their genome or the traits they may possess (including the risk of developing diseases or other characteristics). Thus, the term “genomic information” used in Federal Law No. 242-FZ¹⁰ does not correspond to the biological essence of the subject matter of legal protection, lacks universality, and serves specific purposes of this law. Nevertheless, this legislative act clarifies that in the legal sphere, genetic data that can restore an individual’s identity should be regarded as personal.

At the same time, Article 11, Paragraph 1 of the Federal Law “On Personal Data”¹¹ states that “information characterizing physiological and biological features of a person, based on which their identity can be established (biometric personal data), and which is used by the operator to verify the identity of the subject of personal data, may only be processed if written consent from the subject of personal data is obtained.” In this part of the law, the concept of “biometric personal data” is indirectly introduced. Paragraph 2 of Article 11 indirectly specifies what data can be classified as biometric, mentioning, for instance, the processing of biometric personal data in connection with mandatory state

genomic registration.¹² However, the terms “genetic data” and “biometric personal data” cannot be equated, since the latter is broader and also includes fingerprints, facial images, and other types of data.

Currently, the most comprehensive definitions of “genetic data” and “genetic information” are provided in Federal Law No. 643-FZ, which has come into force.¹³ In the document, within the description of the legal framework for the functioning of the Russian State Information System in the field of genetic data — the National Database of Genetic Information — a definition of “genetic data” is introduced as “the information about the genetic information of various biological objects represented in a format suitable for obtaining, organizing, accumulating, storing, refining, using, distributing, and destroying such information.” Additionally, the meaning of the term “genetic information” is clarified as “the sequence of nucleotides in nucleic acid polymers,” which is the most accurate biological definition, considering not only DNA but also RNA. However, it is important to note that the mentioned information system does not include genetic data obtained from humans, which are actually more sensitive than data obtained from other organisms (plants, animals, bacteria, etc.), since such information can be used for identifying individuals and assessing their characteristics. Although the terminological framework used to describe genetic information obtained from different species is the same, the sensitivity of data obtained from humans prevents extrapolation of the provisions of Federal Law No. 643-FZ¹⁴ to databases containing information about human genetics. Currently, genetic information in the context of human research is not viewed as a general concept but is presented as different types of genetic data, completely unrelated to each other, governed by separate legislative acts.

Legal framework for genetic data in Russia

When discussing the circulation of genetic data, it typically refers to the collection, storage, and dissemination of information obtained from individuals, including its transfer abroad. Within the current Russian legal framework, there is no single legislative act regulating the circulation of all types of genetic data that can be obtained from humans. Interaction with specific types of genetic data is regulated by three federal laws: No. 323-FZ¹⁵, No. 242-FZ¹⁶, and No. 152-FZ¹⁷. The respective areas of

⁹ Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with Amendments.

¹⁰ Ibid.

¹¹ Federal Law “On Personal Data” No. 152-FZ dated 27.07.2006, with Amendments.

¹² Ibid.

¹³ Federal Law No. 643-FZ “On Amendments to the Federal Law ‘On State Regulation in the Field of Genetic Engineering Activity’” dated 29.12.2022.

¹⁴ Ibid.

¹⁵ Federal Law No. 323-FZ “On the Fundamentals of Protecting Citizens’ Health in the Russian Federation” dated 21.11.2011, with Amendments.

¹⁶ Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with Amendments.

¹⁷ Federal Law No. 152-FZ “On Personal Data” dated 27.07.2006, with Amendments.

Table 2. Application areas of legislative acts and data types they are applicable to

Methods for acquiring genetic data	Types of genetic information	Ability to identify an individual	Data type from a legal perspective	Regulatory legal act governing the handling of genetic data in this situation
Scientific research	Exome, genome	Possible	Personal data	Federal Law “On Personal Data” No. 152-FZ dated 27.07.2006, with amendments
Commercial testing (“genetic passport”, genetic panels)	Exome, genome	Possible	Personal data	Federal Law “On Personal Data” No. 152-FZ dated 27.07.2006, with amendments
Genomic registration	Short tandem repeats (STRs)	Possible	Personal data	Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with amendments
Clinical testing	Individual positions in the genome or short DNA fragments, DNA methylation marks, individual transcripts	Impossible	Medical information	Federal Law No. 323-FZ “On the Fundamentals of Protecting Citizens’ Health in the Russian Federation” dated 21.11.2011, with amendments

Table compiled by the authors

application of these legislative acts and the types of genetic information they may apply to are summarized in Table 2.

Genetic information as part of personal data

Genomic information is most frequently treated as an example of personal data, with this trend persisting both in Russia and globally. This approach is valid when the obtained genetic data can be used to identify an individual, i.e., retrieved by appropriate methods (Table 1). In this context, cases involving the acquisition of complete (genome) or nearly complete (exome) DNA sequences can be considered, where such research is conducted either for scientific purposes or at the request of an individual through commercial entities offering genetic testing services aimed at finding relatives, determining lineage, creating a “genetic passport,” and assessing genetic risks across extensive panels that simultaneously examine a large number of genetic loci. The results of DNA sequencing are processed in accordance with Federal Law No. 152-FZ¹⁸, which aims to protect citizens’ rights and freedoms when their personal data is handled, including respecting their privacy. According

to this law, every operator of personal data must obtain written consent from the data subject for processing actions, including collecting, recording, organizing, accumulating, storing, updating (modifying), retrieving, using, transmitting (disseminating, granting access), depersonalizing, blocking, and fully deleting personal data.

In October 2019, the State Duma passed in the first reading a bill proposing amendments to Article 11 of the Federal Law “On Personal Data”¹⁹ concerning the processing of biometric personal data. The bill sought to strengthen the protection of genetic data obtained from individuals. The authors of the bill also highlighted gaps in the legislation “regarding the protection of information about a person obtained from their biomaterial, which contains genetic information that can provide additional insights (about health status, nutrition, lifestyle, behavioral traits, sensitivity to pharmaceutical drugs or allergens, and other individual characteristics).” However, in 2023, the State Duma of the Russian Federation rejected this bill in the second reading.²⁰

In scientific research, each participant signs a consent form agreeing to participate in the study and permitting the processing of personal data, thereby authorizing the operator (research institution) to carry out

¹⁸ Federal Law No. 152-FZ “On Personal Data” dated 27.07.2006, with Amendments.

¹⁹ Ibid.

²⁰ Bill No. 74402-7 on Amendments to Article 11 of the Federal Law “On Personal Data” Regarding Processing of Biometric Personal Data.

a full range of activities with all provided information, including genetic data, within the declared research objectives. Often overlooked is the fact that expanding research objectives, performing new genetic analyses, or publishing such results (either raw genetic data in anonymized form or aggregated statistical analysis results) may necessitate updating the signed consent. In the case of commercial genetic testing, the terms of personal data processing are typically outlined in the Privacy Policy, which participants accept, automatically granting consent for processing genetic data. It is important to note that accepting the Privacy Policy often implicitly grants permission for sharing genetic test results for scientific purposes (mandatory in anonymized form).

State genomic registration

The use of genetic data for state genomic registration also implies treating this information as personal data and is regulated by Federal Law No. 242.²¹ Under this law, the operators responsible for collecting the “genomic information database” and the specific purposes of registration are clearly defined. Thus, “genomic information” is used for crime prevention, detection, and investigation; identifying perpetrators; searching for missing Russian citizens, foreign nationals, and stateless individuals residing or temporarily staying in Russia; identifying deceased individuals whose bodies cannot be recognized by other means; and establishing familial relationships of searched-for individuals. This information is stored for approximately 70 years before being destroyed (Articles 12 and 16 of the Federal Law²²).

Processing rules for the collected data are established by the Ministry of Internal Affairs of the Russian Federation. Accounting, storage, and classification of genetic data obtained during compulsory state genomic registration of convicted individuals serving sentences are managed by the Federal State Institution “Expert Criminalistic Center of the Ministry of Internal Affairs of the Russian Federation” through the creation and maintenance of a federal genomic information database. Currently, genomic registration is mainly conducted using STR typing by licensed laboratories.

Furthermore, on November 7, 2024, amendments to migration-related legislation concerning

genomic registration of foreign citizens and stateless individuals came into effect. Federal Law No. 253²³ “On Amendments to Certain Legislative Acts of the Russian Federation,” dated August 8, 2024²⁴, introduced adjustments to the Federal Law, stipulating that migrants may undergo voluntary genomic registration. The law permits migrant identification using the corresponding genomic database of the Ministry of Internal Affairs. Authorized parties, including courts, investigative agencies, inquiry bodies, and departments conducting operative-search activities, can utilize the collected genomic information database (Article 15, paragraph 1). This database can also be transferred overseas provided that there are relevant international agreements (Article 15, paragraph 2). Presidential directives issued following a meeting of the Council for Civil Society and Human Rights Development on February 7, 2025, mandate an assessment of the feasibility and appropriateness of implementing mandatory genomic registration for military personnel.²⁵ Responsibility for evaluating the necessity of the procedure and preparing proposals for necessary legislative amendments falls jointly on the Russian Defense Ministry and other agencies, including the Ministry of Internal Affairs as the primary operator of the database. Thus, the Federal Law “On State Genomic Registration”²⁶ addresses a specific scenario of using genetic information for the needs of the Ministry of Internal Affairs and cannot be extended to other instances of collecting and processing genetic data.

Genetic data as part of medical information

Genetic data is not always considered personal data [10]. When conducting clinical genetic tests to evaluate the inherent risks of developing various diseases, it is possible to determine the sequence of DNA regions that are insufficient for identifying an individual (individual genome locations or short DNA fragments). It is important to clarify that, in the event of genetic testing in a medical facility and signing a contract for “Provision of Medical Services.” The information gathered during the service is deemed medical, and its processing is regulated by Federal Law No. 323-FZ,²⁷ although the document does not introduce the concepts of “gene,” “genome,” or “genetic information” [11]. In this case, genetic information cannot be freely distributed, as constituting medical confidentiality (Article 13 of Federal Law No. 323²⁸). It

²¹ Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with Amendments.

²² Ibid.

²³ Federal Law No. 253 “On Amendments to Certain Legislative Acts of the Russian Federation” dated 08.08.2024.

²⁴ Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with Amendments.

²⁵ List of Directives Following the Meeting of the Council for Civil Society Development and Human Rights Approved by the President of the Russian Federation on 07.02.2025.

²⁶ Federal Law No. 242-FZ “On State Genomic Registration in the Russian Federation” dated 03.12.2008, with Amendments.

²⁷ Federal Law No. 323-FZ “On the Fundamentals of Protecting Citizens’ Health in the Russian Federation” dated 21.11.2011, with Amendments.

²⁸ Ibid.

should not be disclosed to third parties without patient consent (with exceptions, including requests from investigative and prosecutorial authorities) [12]. Use of data for scientific purposes and publication in scientific journals is permissible only with the patient's explicit consent.

Neither the procedure for collecting, nor procedures for processing and analyzing genetic information, are explicitly described in the legislative acts mentioned above. However, it is implied that the transfer of biological samples for obtaining genetic data is voluntary, except for special cases related to registration, such as certain prisoners.

In summary, Russian legislation is currently lacking a document that could comprehensively regulate the circulation of genetic information obtained from humans. In the context of growing importance of genetic research and genetic technologies in Russia, it appears highly relevant to establish working groups advocating legislative initiatives in this area. For example, a working group on regulatory legal governance in the field of genetic technologies has been operating under the auspices of the Federal Scientific and Technical Program for the Development of Genetic Technologies for 2019–2030 with the purpose of discussing current legal initiatives in genetics. The Kutafin Moscow State Law University hosts a structural unit — the Scientific and Educational Center for Legal Support of Bioeconomy and Genetic Technologies — whose mission includes preparing proposals for improving Russian legislation in the field of development and application of genetic technologies. Following extensive discussions in November 2024, these groups proposed first draft bills that predominantly regulate the application of genetic technologies in agriculture, barely touching upon the biomedical direction.

International practices in legal regulation of genetic data handling

As mentioned earlier, genetic information is classified as personal data and is regulated by relevant laws in most countries worldwide [13]. However, due to the need for a more thorough approach to protecting genetic information, there is a global trend toward developing specialized legislative acts [14]. For instance, in 2018, the European Union (EU) adopted the General Data

Protection Regulation (GDPR)²⁹. The Regulation, firstly, defines genetic information as a type of personal data, and secondly, establishes a strict confidentiality regime for its handling [15].

Genetic research, as well as the storage and processing of genetic data in different EU countries, is regulated by local personal data laws, with significant variability noted in the legal frameworks³⁰ [16]. In some countries, specific laws on genetic research have been adopted, defining which actions, under what conditions, and for what purposes can be carried out during genetic research. For example, in Portugal, the Law No. 12/2005 of 26 January on Personal Genetic Information and Health Information³¹ protects the confidentiality of genetic information. According to this document, access to genetic data is limited to a narrow circle of individuals with the highest level of authorization. The law also provides a definition for genetic databases and establishes the legal procedure for their creation. Furthermore, this document restricts the availability of genetic testing and prohibits discrimination against individuals based on genetic information/the fact of having a genetic disease, which also applies to the activities of insurance companies.

The law also regulates the conduct of scientific research on the human genome and the establishment of biobanks.

In Hungary, “XXI Law on the Protection of Human Genetic Data, on the Rules of Human Genetic Testing and Research and Operation of Biobanks” (2008) has come into force [17]. This law aims to protect genetic information, establishes procedures for the processing of genetic data, sets conditions for the use of this information in scientific research, and regulates the rules for the creation and operation of biobanks.

Specialized laws on genetic research have also been adopted in a number of other countries, including Germany,³² Spain,³³ etc. Furthermore, many governments have enacted a series of specific regulatory legal documents for the application of genetic technologies in humans. These include so-called embryo protection laws (e.g., in Germany³⁴), which define the permissible boundaries for research and genetic testing on embryos and impose a ban on modifications of the germline.

It is noteworthy that many countries focus on developing legislative norms and regulating the establishment

²⁹ Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 Apr. 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). URL: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0679>

³⁰ JRC F7 — Knowledge Health and Consumer Safety, Overview of EU National Legislation on Genomics; 2018. URL: https://www.academia.edu/86948002/Overview_of_EU_National_Legislation_on_Genomics

³¹ Law No. 12/2005 of 26 Jan. on Personal Genetic Information and Health Information. URL: https://www.eshg.org/fileadmin/www.eshg.org/documents/Europe/LegalWS/Portugal_Law-UnofficialEnglishTranslation.pdf

³² Genetic Testing Act (31.07.2009) No. 50/2009 «Gesetz über genetische Untersuchungen bei Menschen (Gendiagnostikgesetz — GenDG)». URL: <http://www.gesetze-im-internet.de/gendg/BJNR252900009.html>

³³ Lacadena J-R. The 14/2007 Law on Biomedical Research: comments on ethical and scientific aspects. *Revista de Derecho y Genoma Humano*. 2007;(27):13–35.

³⁴ Gesetz zum Schutz von Embryonen vom 13.12.1990. URL: <http://www.gesetze-im-internet.de/eschg/index.html>

of biobanks, which limits the range of entities permitted to collect, store, and process genetic information. Biobanks that obtain special authorization operate for specific purposes and under the strict supervision of state authorities. For instance, in Latvia, prior to the launch of the national project,³⁵ the “Human Genome Research Law”³⁶ was adopted, which is distinguished by a greater clarity and a comprehensive approach. Specifically, it stipulates that the use of the database is permitted only for scientific purposes, disease research, and population health studies. Similarly, in Estonia, a separate law was developed to regulate the operations of the national Estonian Biobank and human gene research.³⁷ The main operator of the biobank is defined as the University of Tartu, which may delegate its rights to national organizations that have obtained the corresponding authorization.

In the United States, there is no single federal law regulating all aspects of genomic research. Specialized acts that govern specific procedures for ensuring the safety of human genome research pertain to the protection of personal genetic information, provision of genetic testing services, and prohibition of genetic discrimination. It is important to note that in the United States, the vast majority of organizations conducting genomic research are non-governmental, and oversight of their activities is carried out in accordance with state-level local legislation. Some states have their own laws that include provisions on genome confidentiality, providing additional protection for genetic information (Genome Statute and Legislation Database³⁸). Among the states that regulate the conduct of genetic tests or the obtaining of genetic information, 21 permit the research use of genetic information under specific conditions that eliminate the possibility of personal identification. For instance, the genetic data protection law enacted in California³⁹ defines the types of data that companies performing genetic testing are allowed to process.

The primary law in the United States for the protection of genetic data is the Federal Health Insurance Portability and Accountability Act (HIPAA).⁴⁰ Since 2013, a provision has been in effect stating that genetic information holds the status of Protected Health Information (PHI). However, commercial genetic testing

conducted by private companies such as 23andMe and Ancestry is not considered a healthcare activity and, therefore, does not fall under the purview of HIPAA.

The most important specialized regulatory act governing the handling of genetic information in the United States is the Genetic Information Nondiscrimination Act (GINA), enacted in 2008⁴¹. It aims to protect the American population from genetic discrimination in the areas of health insurance and employment. The act also includes provisions concerning the conduct of genetic and genomic research. It outlines the obligations of research organizations to inform participants about potential consequences and measures taken to ensure confidentiality.

In recent years, China has been actively developing its regulatory framework to govern the management of genomic information.⁴² In 2019, with the aim of strengthening the protection of the population's genetic data and tightening control in this field, the “Regulations on the Management of Human Genetic Resources” were adopted⁴³. According to these regulations, genetic resources are defined as materials (organs, tissues, cells) containing human genes and data obtained during genetic research. It is noteworthy that the authority to regulate the field of genomic research is vested in the Ministry of Science and Technology of the State Council of China (Article 4 of the PRC “Regulations on the Management of Human Genetic Resources”). Thus, in China, it is the state that is responsible for the rational use of genetic data in scientific research or medical activities [18]. It is also important to note that the collection, storage, use, and transfer of genetic materials by foreign organizations are prohibited, as well as the commercial use of human genetic material. Although the Chinese approach to ensuring the security of genetic research largely resembles global practices (mandatory obtaining of voluntary informed consent), human genome research in China is subject to stricter state control.

Thus, specialized regulatory legal acts in the field of genetic information protection are being actively developed and adopted in many countries worldwide. It can be seen that the introduction of new legislative acts alongside general legal instruments is largely connected

³⁵ Genome database of Latvian population. URL: <https://genomadatabase.lv/en/>

³⁶ Human Genome Research Law (with amendments to 02.11.2017). URL: <https://www.vvc.gov.lv/en/laws-and-regulations-republic-latvia-english/human-genome-research-law-amendments-02112017>

³⁷ Human Genes Research Act. URL: <https://www.riigiteataja.ee/en/eli/508042019001/consolidate>

³⁸ Genome Statute and Legislation Database. URL: <https://www.genome.gov/about-genomics/policy-issues/Genome-Statute-Legislation-Database>

³⁹ Senate Bill No. 41 «Genetic Information Privacy Act». URL: https://leginfo.ca.gov/faces/billTextClient.xhtml?bill_id=202120220SB41

⁴⁰ Public Law 104–191–Aug.21, 1996 «Health Insurance Portability and Accountability Act of 1996». URL: <https://www.govinfo.gov/content/pkg/PLAW-104publ191/pdf/PLAW-104publ191.pdf>

⁴¹ The Genetic Information Nondiscrimination Act of 2008. URL: <https://www.federalregister.gov/documents/2015/10/30/2015-27734/genetic-information-nondiscrimination-act-of-2008>

⁴² Gulyaeva EE. Features of Approaches to Legal Regulation of Ensuring Biological Safety in the USA and PRC. URL: <https://www.dipacademy.ru/blog-ekspertov-diplomaticheskoj-akademii/avtory-bloga/gulyaeva-ee/osobennosti-podhodov-k-pravovomu-regulirovaniyu-obespecheniya-biologicheskoy-bezopasnosti-v-ssha-i-knr/>

⁴³ China Regulation No. 717 “On Administration of Human Genetic Resources”; 2019. URL: http://www.gov.cn/zhengce/content/2019-06/10/content_5398829.htm

with the establishment of biobanks and genetic information databases.

Genetic databases as an object of legal protection

With the increasing volume of accumulated genetic information, the subject of discussion has extended to include the legal regulation not only of the handling of individual genetic data but also of the creation and use of genetic databases containing research results from large numbers of people. The world's largest biobanks possess genetic data derived from various biological samples, with the number of included participants ranging from several hundred thousand to several million.

For instance, the UK Biobank,⁴⁴ which aims to improve the prevention, diagnosis, and treatment of a wide range of diseases, includes 500,000 participants and currently represents a powerful resource for the scientific community. The Chinese Kadoorie Biobank (Kadoorie Study of Chronic Disease in China, KSCDC⁴⁵) includes over 510,000 participants; the Finnish FinnGen⁴⁶ project targets 500,000 participants; and the Estonian Biobank⁴⁷ aims for 200,000 participants. The US "All of Us" Biobank⁴⁸ expects to reach a cohort of one million or more volunteers within its broad-spectrum disease research program. The Shanghai-based commercial Zhangjiang Biobank⁴⁹ has announced a target of 10 million samples. The Zhangjiang Biobank utilizes integrated high-throughput research platforms for the analysis of genomic, transcriptomic, and proteomic data to develop tools for the diagnosis and early screening of various diseases.

Most biobanks are involved in nationally government-funded healthcare research. As noted earlier, the establishment of large biobanks often stimulates the development of a regulatory framework that governs their operations and ensures data protection [19]. However, to date, genetic information databases have been established and continue to be expanded not only within specialized biobanks but also across various scientific, medical, and commercial organizations, including in the Russian Federation.

Thus, the creation of Russian bioinformatic and genetic databases is one of the goals of the Federal Scientific and Technical Program for the Development of

Genetic Technologies for 2019–2030⁵⁰. Consequently, their number will steadily increase.

The distinction between a genetic database and the medical and registry information discussed earlier lies in the complete anonymization of the stored information. Databases collected within research projects are not intended to store genetic and other personal data jointly. However, at present, this approach is established more at the level of ethics than legislation. Furthermore, genetic databases represent so-called "big data" arrays — vast amounts of information whose secure storage requires specialized tools and a developed technological infrastructure. The latter is also important because, even when this information is inaccessible to end-users such as research institutions and biomedical specialists, the database operators often possess not only a wide range of supplementary information about each individual whose genome is available but also other data that can unequivocally reveal a substantial layer of information and identify both the individual and potentially their relatives.

Therefore, the protection of genetic databases must be organized at a high technological level and have a clear legislative algorithm for safeguarding. Currently, legislative regulation exists for only one specific database — the "National Genetic Information Database,"⁵¹ which does not collect genetic data obtained directly from humans. Given the anonymized nature of the data collected in such databases, the protection regimes inherent to personal or medical data cannot be applied to them. Consequently, they must occupy a distinct position within the legal framework.

It is important to note that there exist other types of databases which collect information about the distribution of genetic variants within specific populations (for example, dbSNP⁵² — the database of the US National Center for Biotechnology Information), rather than about individuals. In Russia, an example of such a database is the Database of Population Frequencies of Genetic Variants of the Russian Federation Population (Genetic Data Base, GDB)⁵³, published in October 2024, whose operator is the Centre for Strategic Planning of the FMBA.

This database contains information on the frequency of occurrence of over 550 million genetic variants, obtained from whole-genome sequencing data

⁴⁴ UK Biobank. URL: <https://www.ukbiobank.ac.uk>

⁴⁵ China Kadoorie Biobank. URL: <https://www.ckbiobank.org>

⁴⁶ FINNGEN. URL: <https://www.finngen.fi/en>

⁴⁷ EstBB. URL: <https://genomics.ut.ee/en/content/estonian-biobank>

⁴⁸ The All of Us Research Program. URL: <https://allofus.nih.gov/>

⁴⁹ Shanghai Zhangjiang Biobank.

⁵⁰ Resolution of the Government of the Russian Federation No. 479 dated 22.04.2019.

⁵¹ Federal Law No. 643-FZ "On Amendments to the Federal Law 'On State Regulation in the Field of Genetic Engineering Activity'" dated 29.12.2022.

⁵² dbSNP. URL: <https://www.ncbi.nlm.nih.gov/snp/>

⁵³ Population Frequency Database of Genetic Variants of the Russian Population. FMBA of Russia. App Version 1.0.3 dated 05.12.2024. Database Version 59.1 dated 03.10.2024. URL: <https://gdbpop.nir.cspfmba.ru/>

with over 30x coverage from a sample of 120 thousand individuals collected across 85 regions of the Russian Federation. Despite using the results of human whole-genome sequencing, the data compiled in the GDB are fully depersonalized. The epidemiological study conducted as part of the GDB project ensured complete participant anonymity, and the sample collection and delivery protocol eliminated any possibility of identification. Furthermore, the database itself does not contain data on the linkage (co-occurrence in an individual) of genetic variants, thus not revealing which genetic characteristics are specific to a particular person. Instead, it provides aggregated statistical information on the frequency of genetic variants within the Russian population.

Databases, such as the GDB, cannot be used to identify the specific individuals whose data contributed to their creation. However, they contain a vast amount of information about the population structure of the Russian Federation: the proportion of carriers of pathogenic variants, the distribution of frequencies of various mutations, ethnic composition, and other details that can be gleaned from genome analysis.

Consequently, the issue of regulating the creation, storage, and distribution of genetic databases remains to be resolved. Legal discussion has proposed solutions regarding the need to apply a secrecy regime to genetic big data to exert greater control [20]. In this article, the authors proposed “drawing an analogy with the existing regime for geodetic, topographic, and cartographic information, where some data is classified as a state secret, and some as official information with limited distribution” [20].

However, it is crucial to consider that genetic information databases hold significant value for clinicians and the scientific community. Equating genetic data to a state secret would impose severe restrictions on scientific research and the development of biotechnology as a whole. It would also limit access to information necessary for physicians to make informed decisions (for instance, regarding the prevalence of a particular variant in the population), consequently leading to a decline in the quality of medical care and a slowdown in the overall progress of medicine.

Thus, it is necessary to distinguish between approaches to ensuring the protection and confidentiality of genetic information of a particular individual within healthcare delivery or personal use and the protection of genetic information databases containing

anonymized data of individuals for research and clinical purposes [21]. For scientific research, freedom to study genetic information should be ensured, rather than freedom to use and disseminate such information without constraints [22, 23].

CONCLUSION

In Russia, there currently exists a significant dissonance between the progress in genetic technologies and the legislation regulating the resulting data. Thus, neither unified law regarding genetic data, nor subordinate legal acts regulating this issue, nor commissions overseeing this process have been created. The absence of a unified conceptual framework and the multitude of grey areas in the legal regulation of various types of genetic data could lead to serious legal problems, not to mention ethical, deontological, and other issues beyond the scope of the current discussion.

Meanwhile, the urgency of this matter is steadily increasing: each year sees the emergence of more Federal-scale genetic projects. Consequently, legislation governing the relations arising in this context is gradually beginning to develop. As noted in this article, we are currently witnessing the emergence of new legislative initiatives in the field of genetic information management. As researchers, we support this observed trend. The development of such documents must necessarily account for all the shortcomings of existing approaches to legal regulation in this field, identified in our research.

The main directions for development could be as follows:

- defining the types of genetic data subject to legislative regulation and incorporating the relevant concepts into legislative acts;
- updating legislative acts in accordance with advances in scientific and technological progress in the field of genetics;
- establishing the legal regime for databases containing genetic information obtained from humans;
- defining the legal regime for data obtained in the course of scientific research.

Considering global legislative experience and involving leading scientists in the fields of genetics and biotechnology will enable the development and adoption of legislative acts that align with the knowledge of modern biomedical science and regulate relations in new areas of law. This, in turn, will foster the technological development of Russia as a whole.

References

1. Clayton E, Evans B, Hazel J, Rothstein M. The law of genetic privacy: applications, implications, and limitations. *Journal of Law and the Biosciences*. 2019;6(1):1–36. <https://doi.org/10.1093/jlb/lz007>
2. Hallinan D, Friedewald M, De Hert P. Genetic Data and the Data Protection Regulation: Anonymity, multiple subjects, sensitivity and a prohibitory logic regarding genetic data? *Computer Law & Security Review*. 2013;24(4):317–29. <https://doi.org/10.1016/j.clsr.2013.05.013>
3. Semenovich AE. Legal Protection of Genetic and Genomic Information in Genomic Legal Relationship: A Civil Law Aspect. *Lex Genetica*. 2024;3(3):93–106 (In Russ.). <https://doi.org/10.17803/lexgen-2024-3-3-93-106>
4. Erlich HA, Arnheim N. Genetic analysis using the polymerase chain reaction. *Annual Review of Genetics*. 1992;26:479–506. <https://doi.org/10.1146/annurev.ge.26.120192.002403>
5. McDonald C, Taylor D, Linacre A. PCR in Forensic Science: A Critical Review. *Genes*. 2024;15(4):438. <https://doi.org/10.3390/genes15040438>
6. França LTC, Carrilho E, Kist TBL. A review of DNA sequencing techniques. *Quarterly Reviews of Biophysics*. 2002;35(2):169–200. <https://doi.org/10.1017/S0033583502003797>
7. Mardis ER. Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics*. 2008;9:387–402. <https://doi.org/10.1146/annurev.genom.9.081307.164359>
8. Wang Y, Zhao Y, Bollas A, Wang Y, Au KF. Nanopore sequencing technology, bioinformatics and applications. *Nature Biotechnology*. 2021;39(11):1348–65. <https://doi.org/10.1038/s41587-021-01108-x>
9. Booth SA, Drebot MA, Tipples GA, Ng LK. Application of DNA array technology for diagnostic microbiology. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 2000;11(6):291–4. <https://doi.org/10.1155/2000/127160>
10. Rahnasto J. Genetic data are not always personal—disaggregating the identifiability and sensitivity of genetic data. *Journal of Law and the Biosciences*. 2023;10(2):1–45. <https://doi.org/10.1093/jlb/lzad029>
11. Rassolov IM, Chubukova SG. Protection of genetic data in genetic testing and gene-therapy treatment: IT law aspects. *Actual Problems of Russian Law*. 2020;15(5):65–72 (In Russ.). <https://doi.org/10.17803/1994-1471.2020.114.5.065-072>
12. Moskovkina EK. Patient's Privacy and Relatives' Rights in Genetic Research. *Lex Genetica*. 2023;2(2):53–73 (In Russ.). <https://doi.org/10.17803/lexgen-2023-2-2-53-73>
13. Crowell SF. An analysis of legal doctrines and regulations for genetic privacy. *Studies in Law and Justice*. 2023;2(2):32–6. <https://doi.org/10.56397/SLJ.2023.06.05>
14. Kosilkin SV. Principles of International Legal Regulation of Genetic Research and Legislation of the Russian Federation. *Lex Genetica*. 2023;2(2):34–52 (In Russ.). <https://doi.org/10.17803/lexgen-2023-2-2-34-52>
15. Olimid A, Rogozea L, Olimid D. Ethical approach to the genetic, biometric and health data protection and processing in the new EU General Data Protection Regulation (2018). *Romanian Journal of Morphology and Embryology*. 2018;59(2):631–6.
16. Trikoz EN, Gulyaeva EE. The Protection of Human Genetic Information in the EU: Ethical, Constitutional and Criminal Law Aspects. *Lex Genetica*. 2023;2(1):24–44 (In Russ.). <https://doi.org/10.17803/lexgen-2023-2-1-24-44>
17. Sandor J, Bard P. *The Legal Regulation of Biobanks — National Report: Hungary*. Budapest; 2009.
18. Madej M, Karpiński P, Akopyan H, Witt M, Sasiadek M. Genetic data protection as an indispensable element of genomic medicine development. *Polish Archives of Internal Medicine*. 2023;133(2):16425. <https://doi.org/10.20452/pamw.16425>
19. Kubyshev AV. Regulation of Bioresource Centers and Biological Collections at the Level of International Organizations. *Lex Genetica*. 2024;3(4):107–12 (In Russ.). <https://doi.org/10.17803/lexgen-2024-3-4-107-112>
20. Lisachenko AV. The legal regime of “big genomic data”: pros and cons of free circulation. *Russian Juridical Journal*. 2022;2(143):140–51 (In Russ.). https://doi.org/10.34076/20713797_2022_2_140
21. Ayusheeva IZ. Civil Regulation of Genomic Data Sharing. *Lex Genetica*. 2024;3(2):54–73 (In Russ.). <https://doi.org/10.17803/lexgen-2024-3-2-54-73>
22. Chubukova SG. Legal problems of genetic information protection: a subjective approach. *Courier of Kutafin Moscow State Law University (MSAL)*. 2020;5(5):96–103 (In Russ.). <https://doi.org/10.17803/2311-5998.2020.69.5.096-103>
23. Lapaeva VV. Legal support for the creation and use of genetic technologies in medicine. *Medical Genetics*. 2020;19(10):77–8 (In Russ.). <https://doi.org/10.25557/2073-7998.2020.10.77-78>

Authors' contribution. All authors confirm that they meet the ICMJE criteria for authorship. The greatest contribution is as follows: Daria A. Kashtanova — development of the research methodology and concept, scientific supervision, creation and editing of the manuscript; Aleksandra A. Mamchur — collection and analysis of scientific literature, generalization of results and systematization of data, writing — creation of a draft manuscript (section “Types of genetic information subject to legal regulation”), editing of the manuscript; Anna Yu. Yakovchik — collection and analysis of the regulatory framework from foreign sources, writing — creation of a draft and editing of the manuscript (section “Foreign experience in legal regulation of the handling of genetic information”); Aram R. Grigoryan — collection and analysis of the regulatory framework from Russian sources, writing — creation of a draft of the manuscript (sections “State genome registration”, “Genetic information as part of personal data”, “Genetic data as part of medical information”); Mikhail V. Ivanov — collection and analysis of the regulatory framework describing the regulation of databases, writing — creation of a draft manuscript (section “Databases of genetic data as an object of legal protection”); Sergey I. Mitrofanov — editing of the manuscript (sections “Types of genetic information subject to legal regulation”, “Databases of genetic data as an object of legal protection”); Irina N. Svidovskaya — writing, reviewing and editing the manuscript; Vladimir S. Yudin — approving the final text of the manuscript; Anton A. Keskinov — directing and coordinating the research; Sergey M. Yudin — project administration.

AUTHORS

Daria A. Kashtanova, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-8977-4384>
DKashtanova@cspfmmba.ru

Aleksandra A. Mamchur
<https://orcid.org/0000-0002-6025-7663>
AMamchur@cspfmmba.ru

Anna Yu. Yakovchik
AYakovchik@cspfmmba.ru

Aram R. Grigoryan
AGrigoryan@cspfmmba.ru

Mikhail V. Ivanov
<https://orcid.org/0009-0004-7070-5636>
Mivanov@cspfmmba.ru

Sergey I. Mitrofanov
<https://orcid.org/0000-0003-0358-0568>
Mitrofanov@cspfmmba.ru

Irina N. Svidovskaya
Svidovskaya@cspmz.ru

Vladimir S. Yudin, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-9199-6258>
VYudin@cspfmmba.ru

Anton A. Keskinov, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7378-983X>
Keskinov@cspfmmba.ru

Sergey M. Yudin, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-7942-8004>
info@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-370>



HIDDEN BURDEN OF CHRONIC NON-COMMUNICABLE DISEASES. PART 1: PREVALENCE OF GERMLINE VARIANTS ASSOCIATED WITH CARDIOVASCULAR DISEASES IN RUSSIAN POPULATION

Vladimir S. Yudin¹, Aleksandra A. Mamchur¹, Mikhail V. Ivanov¹, Ekaterina A. Nikolaeva¹, Daria A. Kashtanova^{1✉},
Veronika V. Daniel¹, Sergey I. Mitrofanov¹, Anton A. Keskinov¹, Lyudmila P. Kuzmina², Sergey M. Yudin¹

¹ Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical and Biological Agency, Moscow, Russia

² Izmerov Research Institute of Occupational Health, Moscow, Russia

Introduction. Cardiovascular diseases (CVD) are the leading cause of mortality in the Russian Federation. Existing healthcare programs are primarily focused on the treatment of manifest CVD, while the prevalence of genetic risk factors (cardio-germline variants) remains understudied.

Objective. Assessment of the prevalence of pathogenic and likely pathogenic (P/LP) cardio-germline variants in Russian population based on a representative sample.

Materials and methods. The study was conducted using samples from the Genetic Database (GDB) cohort of the Centre for Strategic Planning (FMBA of Russia), comprising 116,794 participants from 86 Russian regions. Whole-genome sequencing (WGS, coverage >30x) of DNA was performed (database of the Centre for Strategic Planning FMBA of Russia). The search and annotation of pathogenic and likely pathogenic (P/LP) variants in 37 genes associated with CVD were performed based on ClinVar. Statistical analysis was conducted using Python (v3.9.12).

Results. P/LP variants in 26 genes were identified in 0.98% (1152) of participants (cumulative allele frequency 0.49%, 424 unique variants). The prevalence by disease groups was as follows: hypercholesterolemia — 0.1704%, structural abnormalities — 0.2218%, arrhythmias — 0.1040%. The most frequent variant was rs5742904 (*APOB* gene, 0.0545%). When strictly considering only variants with the “reviewed by expert panel” level of evidence (Level A), the prevalence was 0.14%. Significant geographical variations were observed (ranging 321–6250 carriers per 100,000 population), with the highest frequencies in the Chechnya, Khakassia, and Bryansk Oblast. No statistically significant decrease in the proportion of carriers was found in the age group over 75 years, confirming the generally low penetrance of these variants. However, within the group over 75 years, a higher number of carriers was observed among female compared to male subjects ($p = 0.013$), which may indicate a greater effect of these variants on male carriers.

Conclusions. The study has identified for the first time a significant “hidden burden” of P/LP cardio-germline variants (~1% carriers) in the Russian population. The substantial geographical variations underscore the importance of a regional approach to healthcare resource planning. The absence of an overall decrease in carrier frequency among the elderly, combined with sex-specific differences in age-related dynamics for certain disease groups, highlights the role of modifying factors and variable penetrance. The data obtained are critical for the development of preventive cardiogenetics and the optimization of the CVD prevention system in Russia.

Keywords: chronic non-communicable diseases; cardiovascular diseases; germline genetic variants; whole-genome sequencing; pathogenic variant; likely pathogenic variant

For citation: Yudin V.S., Mamchur A.A., Nikolaeva E.A., Ivanov M.V., Kashtanova D.A., Daniel V.V., Mitrofanov S.I., Keskinov A.A., Kuzmina L.P., Yudin S.M. Hidden burden of chronic non-communicable diseases. Part 1: Prevalence of germline variants associated with cardiovascular diseases in Russian population. *Extreme Medicine*. 2026;28(1):19–27. <https://doi.org/10.47183/mes.2025-370>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study was approved by the Local Ethical Committee of the Centre for Strategic Planning of the FMBA of Russia (Minutes No. 5 of 28.12.2020; Minutes No. 2 of 01.06.2021). For the creation of the Genetic Database (GDB) of the Centre for Strategic Planning of the FMBA of Russia, voluntary informed consent for participation in the study was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Potential conflict of interest: Sergey M. Yudin is a member of the editorial board of *Extreme Medicine*; Anton A. Keskinov is the scientific editor of *Extreme Medicine*. The other co-authors declare no conflict of interest.

✉ Daria A. Kashtanova DKashtanova@cspfmbs.ru

Received: 6 Aug. 2025 **Revised:** 6 Oct. 2025 **Accepted:** 29 Oct. 2025 **Online first:** 12 Dec. 2025

УДК 631.523:616.1

СКРЫТОЕ БРЕМЯ ХРОНИЧЕСКИХ НЕИНФЕКЦИОННЫХ ЗАБОЛЕВАНИЙ. ЧАСТЬ 1: РАСПРОСТРАНЕННОСТЬ ГЕРМИНАЛЬНЫХ ВАРИАНТОВ, АССОЦИИРОВАННЫХ С СЕРДЕЧНО-СОСУДИСТЫМИ ЗАБОЛЕВАНИЯМИ, СРЕДИ НАСЕЛЕНИЯ РОССИЙСКОЙ ФЕДЕРАЦИИ

В.С. Юдин¹, А.А. Мамчур¹, М.В. Иванов¹, Е.А. Николаева¹, Д.А. Каштанова^{1✉}, В.В. Даниэль¹, С.И. Митрофанов¹,
А.А. Кескинов¹, Л.П. Кузьмина², С.М. Юдин¹

¹ Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

² Научно-исследовательский институт медицины труда им. академика Н.Ф. Измерова, Москва, Россия

© V.S. Yudin, A.A. Mamchur, E.A. Nikolaeva, M.V. Ivanov, D.A. Kashtanova, V.V. Daniel, S.I. Mitrofanov, A.A. Keskinov, L.P. Kuzmina, S.M. Yudin, 2025

Введение. Сердечно-сосудистые заболевания (ССЗ) — ведущая причина смертности в РФ. Существующие программы ориентированы на лечение манифестных ССЗ, в то время как оценка распространенности генетических факторов риска (кардиогерминальных вариантов) остается недостаточной.

Цель. Оценка распространенности патогенных и вероятно патогенных (P/LP) кардиогерминальных вариантов среди населения РФ на репрезентативной выборке.

Материалы и методы. Исследование проведено на образцах из выборки базы данных популяционных частот (GDB) ЦСП ФМБА России, 116 794 участников из 86 регионов РФ. Проведено полногеномное секвенирование (WGS, покрытие >30x) ДНК (база данных ФГБУ «ЦСП» ФМБА России). Поиск и аннотация патогенных и вероятно патогенных (P/LP) вариантов в 37 генах, ассоциированных с ССЗ, выполнены на основе ClinVar. Статистический анализ проведен с использованием Python (v3.9.12).

Результаты. P/LP-варианты в 26 генах выявлены у 0,98% (1152) участников (суммарная частота аллелей 0,49%, 424 уникальных варианта). Распространенность по группам патологий: гиперхолестеринемия — 0,1704%, структурные нарушения — 0,2218%, нарушения ритма — 0,1040%. Наиболее частый вариант — rs5742904 (ген *APOB*; 0,0545%). При строгом учете только вариантов с использованием уровня доказательности «проверено экспертной комиссией» (уровень A) распространенность составила 0,14%. Установлены значимые географические различия (321–6250 носителей на 100 тыс. населения), максимум — в Чеченской Республике, Республике Хакасия, Брянской области. Не обнаружено статистически значимого снижения доли носителей в возрастной группе старше 75 лет, что подтверждает низкую пенетрантность вариантов в целом. При этом внутри группы старше 75 лет среди женщин наблюдали большее число носителей, чем среди мужчин ($p = 0,013$), что может свидетельствовать о большем эффекте данных вариантов на мужчин-носителей.

Выводы. Исследование впервые выявило значимое «скрытое бремя» P/LP кардиогерминальных вариантов (~1% носителей) в российской популяции. Существенные географические различия подчеркивают важность регионального подхода к планированию ресурсов здравоохранения. Отсутствие общего снижения носительства у пожилых в сочетании с половыми различиями в возрастной динамике для отдельных групп патологий подчеркивает роль модифицирующих факторов и различной пенетрантности. Полученные данные критичны для развития превентивной кардиогенетики и оптимизации системы профилактики ССЗ в РФ.

Ключевые слова: хронические неинфекционные заболевания; сердечно-сосудистые заболевания; герминальные генетические варианты; полногеномное секвенирование; патогенный вариант; вероятно патогенный вариант

Для цитирования: Юдин В.С., Мамчур А.А., Иванов М.В., Николаева Е.А., Каштанова Д.А., Даниэль В.В., Митрофанов С.И., Кескинов А.А., Кузьмина Л.П., Юдин С.М. Скрытое бремя хронических неинфекционных заболеваний. Часть 1: распространенность герминальных вариантов, ассоциированных с сердечно-сосудистыми заболеваниями, среди населения Российской Федерации. *Медицина экстремальных ситуаций*. 2026;28(1):19–27. <https://doi.org/10.47183/mes.2025-370>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование одобрено локальным этическим комитетом ФГБУ «ЦСП» ФМБА России (протокол № 5 от 28.12.2020, протокол № 2 от 01.06.2021). Для создания базы данных популяционных частот (GDB) ФГБУ «ЦСП» ФМБА России от всех участников получено добровольное информированное согласие на участие в исследовании, проведенном в соответствии с принципами Хельсинкской декларации.

Потенциальный конфликт интересов: С.М. Юдин — член редакционной коллегии журнала «Медицина экстремальных ситуаций», А.А. Кескинов — научный редактор журнала «Медицина экстремальных ситуаций», остальные авторы заявляют об отсутствии конфликта интересов.

✉ Каштанова Дарья Андреевна DKashtanova@cspfmba.ru

Статья поступила: 06.08.2025 **После доработки:** 06.10.2025 **Принята к публикации:** 29.10.2025 **Online first:** 12.12.2025

INTRODUCTION

Cardiological diseases (CVD) represent one of the major healthcare challenges in the Russian Federation. For a long period of time, they have remained one of the leading causes of mortality.¹ In 2023 alone, mortality from various CVD reached 556.7 per 100,000 population. With the aim of preserving and prolonging the lives of CVD patients, in 2019, the Federal project entitled “Combating Cardiovascular Diseases” was launched. Starting from 2025, this project has become part of the new National Project entitled “Long and Active Life.” It should be mentioned that these programs are aimed primarily at managing already diagnosed CVD. However, for preventive purposes, assessment of not only the prevalence of manifest CVD, but also the risk factors for their development is essential, representing an equally important task for modern epidemiology. The

conventional approach to assessing the disease burden in a particular country is based on clinical data and lifestyle information. However, in the case of CVD, genetic predisposition plays a significant role in determining individual-level risks [1].

The search for genetic variants associated with the development of chronic non-communicable diseases (NCDs) in general, and cardiovascular diseases (CVD) in particular, is a serious challenge in medical genetics. However, recent progress in sequencing technologies has provided information on the association between specific polymorphisms and various clinical conditions. In 2015, with a view to standardizing the assessment of identified genetic variants, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) developed a five-tier classification system [2]. The ACMG/AMP criteria consider various types of data (family segregation,

¹ Mortality from diseases of the circulatory system (per 100,000 population). URL: <https://fedstat.ru/indicator/55382> (access date 21.07.2025).

computational predictions of functional effect, data from reliable sources) and categorize a variant into the following classes: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B). These guidelines have become a global standard, with numerous computational approaches having been proposed to automate their application [3].

Currently, a significant number of cardio-germline genes are known, which can be broadly grouped into clusters based on the CVD type they are associated with. Testing for some of these genes has even entered clinical practice for differential diagnosis. For instance, screening for *LDLR*, *APOB*, and *PCSK9* is recommended as a first-tier investigation for diagnosing familial hypercholesterolemia [4]. In a quarter of individuals with a prolonged QT interval ($QTc > 480$ ms), either rare monogenic variants (3.4%) or a high polygenic risk (21%) were identified, with variants in *KCNQ1* and *KCNH2* significantly prolonging the QTc interval [5]. Titin (*TTN*) variants, associated with cardiac remodeling in healthy individuals, increase the risk of dilated cardiomyopathy (DCM), heart failure (HF), atrial fibrillation (AF), and death by approximately 2.2 times [6]. However, the majority of carriers do not experience these clinical events, indicating the crucial role of triggers. These triggers can be various life events, such as pregnancy, chemotherapy, or intense physical exertion (similar to the case of sudden cardiac death in athletes).

Thus, the mere presence of a genetic variant is insufficient for definitively predicting the risk of CVD development: the majority of currently known pathogenic and likely pathogenic variants exhibit low penetrance. Nevertheless, information about the presence of genetic risk factors can motivate patients to adopt a healthier lifestyle. This information is equally important for the healthcare system. Understanding the genetic burden carried by the population of a specific region allows for forecasting potential disease incidence levels and accounting for the corresponding staffing and financial demands. This fact makes research at the intersection of epidemiology and genetics particularly relevant, enabling the assessment of the prevalence of pathogenic genetic variants both at the level of individual regions and across an entire country.

To date, most clinical studies of genes associated with hereditary CVD have involved patients and families with pre-existing pathologies. In one studied cohort [7] of over 8500 patients referred for cardiac catheterization, pathogenic/likely pathogenic (P/LP) variants in genes associated with cardiomyopathies, arrhythmias, aortopathies, and familial hypercholesterolemia were identified in 4.5% of individuals. However, including only people with already developed cardiovascular pathologies in a study does not permit a reliable assessment of the penetrance of CVD-associated genes. It appears that studies of population cohorts without identified clinical

symptoms of CVD are necessary. Such research is already being actively conducted worldwide. For instance, population screening of 21,846 participants without prior indications for CVD genetic testing (eMERGE-III) revealed 0.6% carriers of P/LP variants in arrhythmia genes; for some of them (0.05%), the diagnosis was established only after a genetic test, and functional studies helped reclassify VUS [8].

This study aims to assess the prevalence of pathogenic and likely pathogenic (P/LP) cardio-germline variants in the population of Russia using a representative sample.

MATERIALS AND METHODS

Study Sample

This study is a continuation of a large-scale epidemiological project initiated in 2019 by the Centre for Strategic Planning of the FMBA of Russia to investigate the prevalence of genetic variants associated with the risk of chronic diseases, including cardiac conditions, among the adult population across 86 federal subjects of the Russian Federation. The study was conducted using a population-based sample from the Genetic Database (GDB) of the Russian Population of the Centre for Strategic Planning of the FMBA of Russia.²

The final randomized representative sample included 116,794 individuals from 86 federal subjects of the Russian Federation. The demographic characteristics of the participants (age and sex distribution) are presented in Figure 1. The median age was 51 [22;73] years for men and 51 [23;75] years for women.

Whole-genome sequencing

DNA was extracted from whole blood samples using the MagAttract HMW DNA Kit (Qiagen, Germany) on an automated robotic station. Whole-genome sequencing libraries were prepared using the Illumina DNA Prep kit (Illumina, USA) according to the manufacturer's instructions. Whole-genome sequencing was performed on the Illumina NovaSeq 6000 platform using the S4 Reagent Kit (300 cycles) (Illumina, USA) for paired-end reads of 2×150 bp. During the demultiplexing stage, sequencing data in BCL format were converted to FASTQ format using Illumina bcl2fastq v2.20. The sequencing run quality was controlled using Illumina Sequencing Analysis Viewer v2.4.7, while the read quality (FASTQ, GZ format) was assessed using the FastQC v0.11.9 bioinformatic tool. Alignment to the reference genome (GRCh38) was performed on the Dragen Bio-IT platform (Illumina, USA). The quality control of alignments (BAM format) was conducted using DRAGEN, FastQC v0.11.9, samtools v1.13, and mosdepth v0.3.1. All samples passed quality control based on key metrics, including

² Genetic Database (GDB). FMBA of Russia. Application v1.1.3 of 17.03.2025. Database v59.1 of 03.10.2024. URL: <https://gdbpop.nir.cspfmba.ru/> (access date 01.08.2025) (In Russ.).

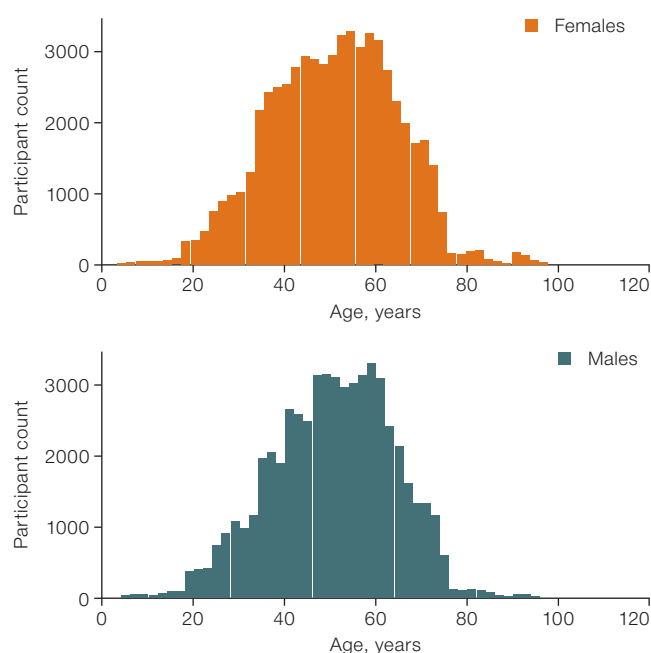


Figure prepared by the authors based on original data

Fig. 1. Age and sex distribution of the study participants

the percentage of duplicated reads and the number of unaligned reads. The final mean genome coverage per sample was at least 30x. Detection of single nucleotide polymorphisms (SNPs) and short insertions/deletions (up to 50 bp) was performed using Strelka2 [9]; only variants passing the PASS filter were selected.

Search for pathogenic and likely pathogenic germline variants associated with cardiovascular diseases (CVD)

A search for pathogenic and likely pathogenic genetic variants associated with pathologies of the cardiovascular system was conducted within the study sample, based on the ClinVar database. The following levels of evidence for the relationship between a genetic variant and the development of the corresponding phenotype, as provided by ClinVar (in descending order of strength), were used for annotating the obtained list of variants:

- Practice guideline
Highest level. Interpretation is based on official clinical guidelines from authoritative professional societies. This is the most reliable data for clinical decision making.
- Expert panel
Very high level. The variant has been thoroughly evaluated and approved by an independent expert group (e.g., within the ClinGen consortium). This also constitutes an extremely reliable basis for clinical action.
- Multiple submitters
High reliability. Multiple laboratories have independently reached the same interpretation of the variant,

with no conflicting data existing. This is a reliable indicator for use in clinical practice.

- Single submitter
Moderate reliability. The interpretation originates from only one laboratory or research group. Such data require caution and verification through scientific publications or other sources.
- No assertion provided
Low reliability. The submitter provided information about the variant but did not offer any clinical interpretation. This information should not be used for clinical decision making.

The variant search was conducted in 37 genes listed in the 2021 review by the American College of Medical Genetics and Genomics (ACMG) [10], associated with the following types of CVD: aortopathies; arrhythmogenic right ventricular cardiomyopathy; catecholaminergic polymorphic ventricular tachycardia; dilated cardiomyopathy; Ehlers-Danlos syndrome; familial hypercholesterolemia; hypertrophic cardiomyopathy; long QT syndrome types 1, 2, and 3; Brugada syndrome.

Accordingly, the variant search was performed across the following genes: *FBN1*, *TGFBR1*, *TGFBR2*, *SMAD3*, *ACTA2*, *MYH11*, *PKP2*, *DSP*, *DSC2*, *TMEM43*, *DSG2*, *RYR2*, *CASQ2*, *TRDN*, *TNNT2*, *LMNA*, *FLNC*, *TTN*, *BAG3*, *DES*, *RBM20*, *TNNC1*, *COL3A1*, *LDLR*, *APOB*, *PCSK9*, *MYH7*, *MYBPC3*, *TNNI3*, *TPM1*, *MYL3*, *ACTC1*, *PRKAG2*, *MYL2*, *KCNQ1*, *KCNH2*, *SCN5A*.

Statistical data processing

Statistical data processing and result visualization were performed using the Python programming language (v3.9.12). Intergroup comparison of the number of carriers of genetic variants was conducted using the chi-square test.

RESULTS

Annotation of variants associated with CVD

In the analyzed sample, carriers of pathogenic and likely pathogenic variants were identified in 26 genes (out of 37 examined) associated with various CVDs, namely: hypercholesterolemia (*APOB*, *LDLR*), structural heart and vascular abnormalities (*BAG3*, *COL3A1*, *DES*, *DSC2*, *DSG2*, *DSP*, *FBN1*, *FLNC*, *LMNA*, *MYBPC3*, *MYH11*, *MYH7*, *MYL2*, *PKP2*, *PRKAG2*, *TMEM43*, *TNNI3*, *TPM1*, *TTN*), and rhythm and conduction disorders (*CASQ2*, *KCNH2*, *KCNQ1*, *SCN5A*, *TRDN*).

At least one variant in these genes was detected in 1152 (0.98%) participants. The number of unique variants identified was 424, with a cumulative frequency in the entire sample of 0.49%. The most frequently encountered variant was rs5742904 (NC_000002.12:g.21006288C>T) in the *APOB* gene, with a frequency of 0.0545%. According to the GDB, this frequency is approximately

consistent with those obtained in other global projects (frequency in gnomAD v4.1.0 — 0.042%, frequency in ALFA — 0.0441%; no statistically significant differences from the Russian population were found). The frequency of variants associated with hypercholesterolemia was 0.1704%, with structural heart and vascular abnormalities — 0.2218%, and with rhythm and conduction disorders — 0.1040%.

The distribution of the identified variants by level of evidence is presented in Figure 2A. It can be noted that none of the found variants had the “practice guideline” level of evidence.

Depending on the selected level of evidence, both the overall proportion of carriers of pathogenic and likely pathogenic variants and the proportion of carriers of variants associated with specific cardiopathologies changed. In total, Level A variants (only those with the “expert panel” level of evidence) were carried by 0.14% of individuals. Level B variants (those with “expert panel” and “multiple submitters, no conflicts” levels of evidence) were carried by 0.8%. Level C variants (those with “expert panel,” “multiple submitters, no conflicts,” and “single submitter” levels of evidence) were characteristic of 0.95% of individuals. Level D included all variants and was characteristic of 0.99% of individuals (the

entire group of carriers of pathogenic and likely pathogenic variants). The distribution of carriers across variant groups according to the level of evidence and associated cardiopathologies is presented in Figure 2B.

It should be noted that the largest number of variants had the “criteria provided, multiple submitters, no conflicts” level of evidence. Consequently, the sharpest increase in the number of carriers was observed when transitioning from Level A to Level B. The addition of further, less substantiated variants only marginally increased the overall proportion of individuals.

It is important to highlight that the prevalence of pathogenic and likely pathogenic variants in a population typically exhibits an age-dependent dynamic. Carriage of these genetic variants is presumed to negatively impact survival and the likelihood of reaching old age. However, our analysis (Fig. 3) revealed some inconsistencies with this hypothesis. Specifically, the proportions of P/LP carriers, regardless of the associated cardiopathology, changed only marginally from one age group to another from a statistical standpoint.

Furthermore, we observed, albeit statistically non-significant, an increase in the proportion of carriers of cardio-germline variants in the oldest age category (over 75 years) compared to the others (Fig. 3). This is

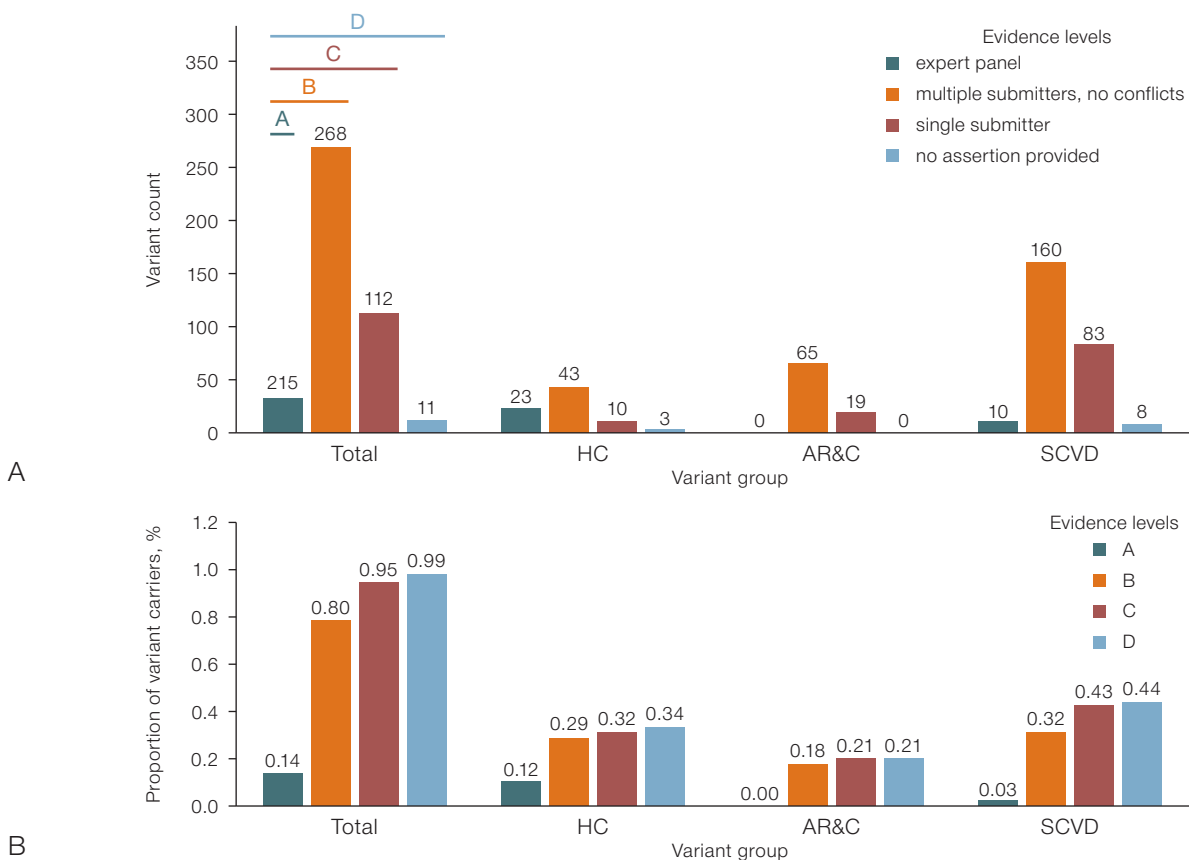


Figure prepared by the authors based on original data

Fig. 2. Annotation of identified variants according to level of evidence: A — number of identified variants with the corresponding level of evidence in each cardiopathology group; B — proportion of carriers of variants associated with different cardiopathologies, stratified by level of evidence; HC — hypercholesterolemia; AR&C — arrhythmias and conduction disorders; SCVD — structural cardiovascular disorders

particularly noticeable for genetic variants associated with familial hypercholesterolemia: in this case, the p -value for intergroup comparison approached the threshold of statistical significance (p -value = 0.065).

Continuing the analysis, we examined the age-related dynamics of cardio-germline variant carriage separately for men and women. For variants associated with hypercholesterolemia, a statistically significant association with age was demonstrated for women (p -value = 0.013). Specifically, they exhibited a significant increase in carrier frequency in the group over 75 years old. Similarly to other groups, women over 75 predominantly carried germline variants in the *APOB* gene. In contrast, among men in this age group, the proportion of carriers of similar variants drops radically: from 0.4% in the 60–74 age group to 0.12% in the over 75 age group (this finding is not statistically significant, p -value = 0.095).

Analysis of the distribution of pathogenic and likely pathogenic variants across the federal subjects of the Russian Federation

The prevalence of cardio-germline variants is associated not only with age but also with the geographical distribution of the Russian population (Fig. 4). The conducted analysis enabled the creation of a map illustrating the frequency of pathogenic and likely pathogenic variants across different regions of the Russian Federation. The highest frequencies are observed in the Chechnya (6250 variants per 100,000 population), Khakassia (2857 per 100,000), Bryansk Oblast (2632 per 100,000), Kurgan Oblast (2,532 per 100,000), and Murmansk Oblast (2,297 per 100,000). The lowest frequencies are found in Novgorod Oblast (472 per 100,000), Pskov Oblast (437 per 100,000), Crimea (407 per 100,000), Arkhangelsk Oblast (367 per 100,000), and Amur Oblast

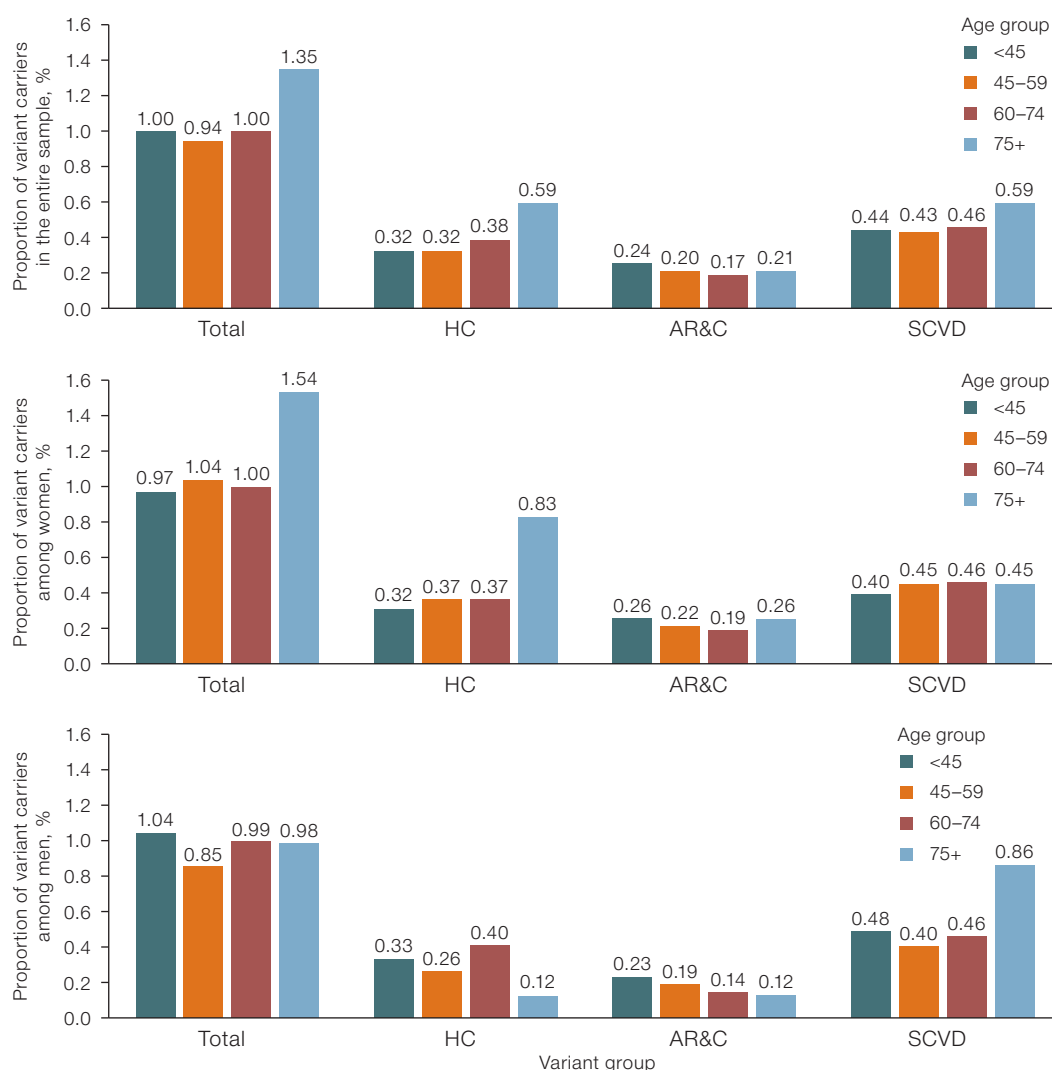


Figure prepared by the authors based on original data

Fig. 3. Changes in the proportion of carriers of cardio-germline variants across different age groups in the entire sample, and separately among men and women: HC — hypercholesterolemia; AR&C – arrhythmias and conduction disorders; SCVD – structural cardiovascular disorders

(321 per 100,000). In Moscow, the prevalence of cardio-germline variants was 1097 per 100,000 population. The overall population frequency is 992 variants per 100,000 population.

DISCUSSION

This large-scale study, bridging epidemiology and genetics, assessed the prevalence of pathogenic/likely pathogenic (P/LP) variants in certain genes associated with cardiovascular diseases within a representative sample of the Russian population ($n = 116,794$). The inclusion of 86 federal subjects of Russia enabled a comprehensive overview reflecting the genetic diversity of a vast and ethnically heterogeneous country, unlike studies confined to specific regions or clinical centers. The inclusion of individuals without pre-selection based on cardiac diagnoses provides an estimate of the “hidden” genetic burden within the population, identifying asymptomatic carriers typically missed by clinical screening. Such research establishes a fundamental basis for understanding the population risk of hereditary cardiovascular diseases (CVD) in Russia, which is essential for planning preventive programs and allocating healthcare resources.

The identification of 0.99% (or ~0.14% using the strictest Level A criterion) carriers of P/LP variants in 26 genes associated with CVD within a clinically unselected population confirms the presence of a significant

“hidden” genetic burden of CVD in Russia. This aligns with data from international biobanks (e.g., eMERGE-III [11]) and underscores that a substantial proportion of individuals at high genetic risk (estimated at over 90%) are asymptomatic at the time of examination, yet remain at risk for developing fatal outcomes [12].

Furthermore, some particularly rare genetic variants may have been potentially missed within the scope of this study, as also evidenced by the absence of eleven initially considered genes in the final list.

The study revealed substantial geographical variation in the prevalence of cardio-germline variants (321–6250 per 100,000 population). This variability likely reflects the ethnic diversity of the Russian Federation and possible “founder effects,” implying an increased frequency of specific pathogenic variants inherited from a common ancestor, or pathogenic variants that are rare or absent in other populations. Despite the large overall sample size, some regions were represented by a relatively small number of individuals, making statistical fluctuations in frequency estimates more pronounced in these areas. Regions where residents are more frequently carriers of pathogenic variants may require enhanced resources for genetic testing, counseling, and cardiological monitoring.

The absence of a statistically significant decrease in the proportion of P/LP variant carriers in the older age groups (over 75 years) provides compelling confirmation of the low penetrance of most known cardio-germline

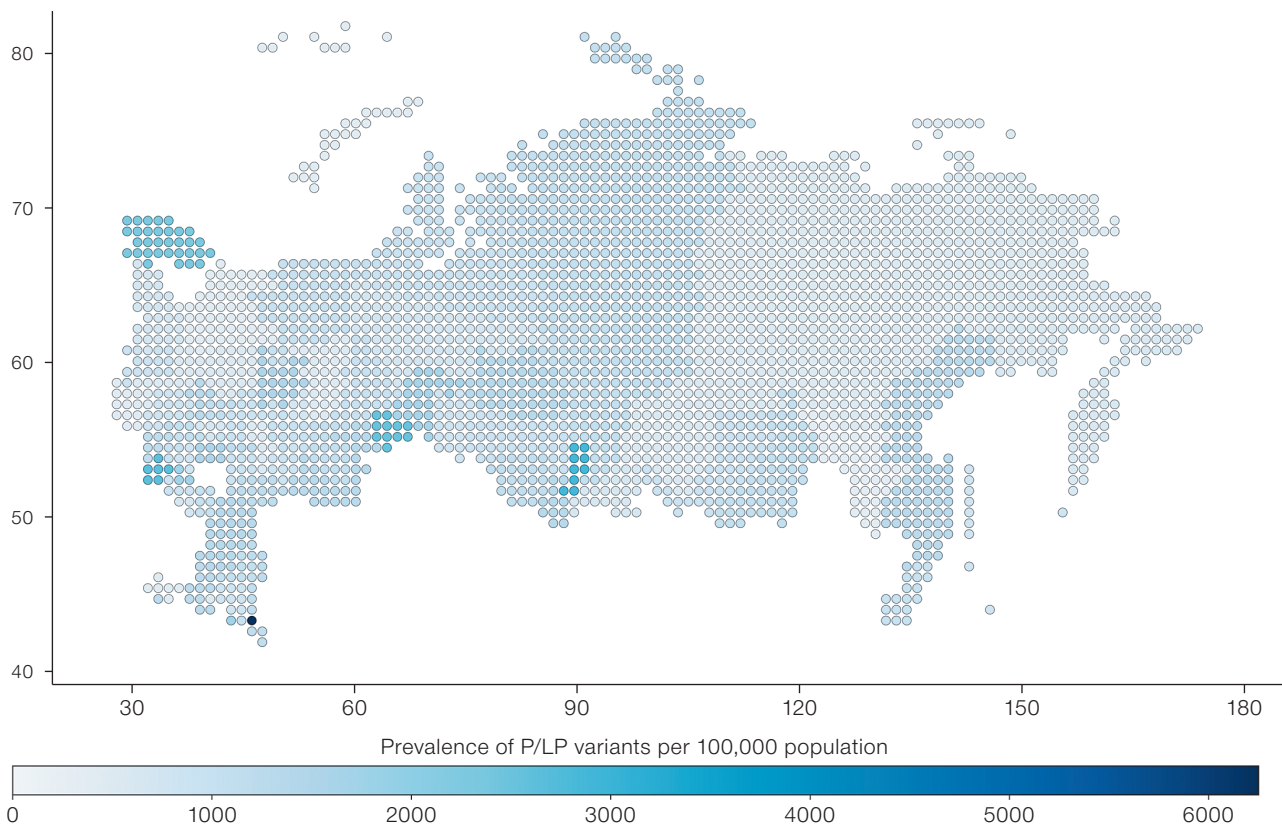


Figure prepared by the authors based on original data

Fig. 4. Prevalence map of cardio-germline variants in the population of the Russian Federation

variants. The presence of a pathogenic allele is not the sole condition for disease development. Its manifestation depends on a complex interplay of genetic and non-genetic factors (age, sex, lifestyle, stress, infections, medication use, and the presence of various triggers).

The high proportion of female carriers of cardio-germline variants in this age group indicates a significantly greater resilience of women to this risk factor compared to men. The higher risk of developing hypercholesterolemia and subsequent ischemic heart disease and myocardial infarction in men is frequently noted in the scientific community, particularly in the context of Familial Hypercholesterolemia (FH) [13, 14]. While differences in the carriage of P/LP variants associated with hypercholesterolemia between men and women are explicable, the observed increase in the proportion of female carriers with age raises questions. Two primary explanations exist for this observation. The first relates to the molecular mechanisms underlying hypercholesterolemia, specifically the accumulation of lipids, particularly LDL-cholesterol. Carriers of P/LP variants associated with hypercholesterolemia are prone to enhanced cholesterol accumulation, which can lead to the development of atherosclerotic lesions in the presence of corresponding lifestyle triggers. However, if an individual manages to avoid developing the disease by a certain age, these very mechanisms might, conversely, become protective against many conditions in old age. For instance, we have previously demonstrated a link between elevated body mass index and lipid levels with a successful aging phenotype in long-livers [15].

The second explanation is related to the geographical distribution of the elderly sample. According to Rosstat data, the elderly population is not uniformly distributed across the country: in Moscow and surrounding regions, the proportion of the population over 65 exceeds 20%, whereas in the Far East and the republics of the North

Caucasus (e.g., Chechnya, Ingushetia), it is less than 10%. Therefore, when examining different age groups, we are simultaneously shifting the regional composition of the sample, which inevitably influences the observed frequency of P/LP variants.

This interplay between the frequency of P/LP variants and the demographic profile of a region could be another reason for the observed geographical heterogeneity presented in Figure 3. This fact further underscores the necessity of simultaneously accounting for multiple factors (both genetic and non-genetic) when assessing risks, both at the level of an individual organism and at the regional level.

Despite the aforementioned low penetrance, carrying P/LP variants significantly increases the risk of CVD compared to the average population risk. Incorporating information about the presence of P/LP variants in each specific patient, along with data on the prevalence of such variants in the population, will enable the development of optimal protocols for preventive medicine. This, in turn, will contribute to improving the quality of life, extending the working-age lifespan, and reducing mortality in Russia.

CONCLUSION

This study provides unique data on the hidden genetic burden associated with the risk of severe hereditary cardiovascular diseases (CVD) in the Russian population. The identified carrier frequency of pathogenic and likely pathogenic variants (up to 0.99%) underscores both the potential of preventive cardiogenetics and the significant challenges on the path to its implementation. Successfully addressing these challenges will enable a transition from merely identifying carriers to actually reducing the incidence and mortality from hereditary CVD in Russia.

References

- Landstrom AP, Chahal AA, Ackerman MJ, Cresci S, Milewicz DM, Morris AA, et al. Interpreting Incidentally Identified Variants in Genes Associated with Heritable Cardiovascular Disease: A Scientific Statement from the American Heart Association. *Circulation: Genomic and Precision Medicine*. 2023;16(2):e000092. <https://doi.org/10.1161/HCG.0000000000000092>
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*. 2015;17(5):405–24. <https://doi.org/10.1038/gim.2015.30>
- Zucca S, Nicora G, De Paoli F, Carta MG, Belazzi R, Magni P, et al. An AI-based approach driven by genotypes and phenotypes to uplift the diagnostic yield of genetic diseases. *Human Genetics*. 2025;144(2–3):159–71. <https://doi.org/10.1007/s00439-023-02638-x>
- Trinder M, Francis GA, Brunham LR. Association of monogenic vs polygenic hypercholesterolemia with risk of atherosclerotic cardiovascular disease. *JAMA Cardiology*. 2020;5:390–9. <https://doi.org/10.1001/jamacardio.2019.5954>
- Nauffal V, Morrill VN, Jurgens SJ, Choi SH, Hall AW, Weng LC, et al. Monogenic and polygenic contributions to QTc prolongation in the population. *Circulation*. 2022;145:1524–33. <https://doi.org/10.1161/CIRCULATIONAHA.121.057261>
- Asatryan B, Murray B, Tadros R, Rieder T, Shah RA, Dabbagh GS, et al. Promise and Peril of a Genotype-First Approach to Mendelian Cardiovascular Disease. *JAMA*. 2024;13(21):e033557. <https://doi.org/10.1161/JAMA.123.033557>
- Abdulrahim JW, Kwee LC, Alenezi F, Sun AY, Baras A, Ajayi TA, et al. Identification of Undetected Monogenic Cardiovascular Disorders. *JACC*. 2020;76(7):797–808. <https://doi.org/10.1016/j.jacc.2020.06.037>
- Glazer AM, Davogustto G, Shaffer CM, Vanoye CG, Desai RR, Farber-Eger EH, et al. Arrhythmia variant asso-

- ciations and reclassifications in the eMERGE-III sequencing study. *Circulation*. 2022;145:877–91.
<https://doi.org/10.1161/CIRCULATIONAHA.121.055562>
9. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Kallberg M, et al. Strelka2: fast and accurate calling of germline and somatic variants. *Nature Methods*. 2018;15:591–4.
<https://doi.org/10.1038/s41592-018-0051-x>
 10. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 2022;24:1407–14.
<https://doi.org/10.1016/j.gim.2022.04.006>
 11. Parikh VN. Promise and Peril of Population Genomics for the Development of Genome-First Approaches in Mendelian Cardiovascular Disease. *Circulation: Genomic and Precision Medicine*. 2021;14(1):e002964.
<https://doi.org/10.1161/CIRCGEN.120.002964>
 12. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, et al. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *The New England Journal of Medicine*. 2016;374(25):2441–52.
<https://doi.org/10.1056/NEJMoa1510687>
 13. Gouni-Berthold I, Laufs U. Special Aspects of Cholesterol Metabolism in Women. *Deutsches Arzteblatt*. 2024;121(12):401–6.
<https://doi.org/10.3238/arztebl.m2024.0063>
 14. Klevmoen M, Mulder JWCM, Roeters van Lennep JE, Holven KB. Sex Differences in Familial Hypercholesterolemia. *Current Atherosclerosis Reports*. 2023;25(11):861–8.
<https://doi.org/10.1007/s11883-023-01155-6>
 15. Kashtanova DA, Taraskina AN, Erema VV, Akopyan AA, Ivanov MV, Strazhesko ID, et al. Analyzing Successful Aging and Longevity: Risk Factors and Health Promoters in 2020 Older Adults. *International Journal of Environmental Research and Public Health*. 2022;19(13):8178.
<https://doi.org/10.3390/ijerph19138178>

Authors' contributions. All authors confirm that they meet the ICMJE authorship criteria. The major contributions are distributed as follows: Vladimir S. Yudin — methodology development and research conceptualization, formulation of scientific objectives, writing, editing, and final approval of the manuscript; Aleksandra A. Mamchur — formal data analysis, summarization and systematization of results, data visualization, manuscript editing; Mikhail V. Ivanov — software development, data administration, data visualization; Ekaterina A. Nikolaeva — summarization of results, drafting the manuscript; Daria A. Kashtanova — scientific supervision, critical revision of the manuscript text; Veronika V. Daniel — data verification, manuscript editing; Sergey I. Mitrofanov — software development, conducting the study; Anton A. Keskinov — research administration; Lyudmila P. Kuzmina — research leadership and coordination, provision of resources, result verification; Sergey M. Yudin — project administration, provision of resources.

AUTHORS

Vladimir S. Yudin, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-9199-6258>
VYudin@cspfmmba.ru

Aleksandra A. Mamchur
<https://orcid.org/0000-0002-6025-7663>
AMamchur@cspfmmba.ru

Mikhail V. Ivanov
<https://orcid.org/0009-0004-7070-5636>
Mivanov@cspfmmba.ru

Ekaterina A. Nikolaeva
<https://orcid.org/0000-0002-7954-2560>
Enikolaeva@cspfmmba.ru

Daria A. Kashtanova, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-8977-4384>
DKashtanova@cspfmmba.ru

Veronika V. Daniel, Cand. Sci. (Med.)
<https://orcid.org/0000-0003-0547-3280>
Vdaniel@cspfmmba.ru

Sergey I. Mitrofanov
<https://orcid.org/0000-0003-0358-0568>
Mitrofanov@cspfmmba.ru

Anton A. Keskinov, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7378-983X>
Keskinov@cspfmmba.ru

Lyudmila P. Kuzmina, Dr. Sci. (Biol.)
<https://orcid.org/0000-0003-3186-8024>
lpkuzmina@mail.ru

Sergey M. Yudin, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-7942-8004>
info@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-366>

HIDDEN BURDEN OF CHRONIC NON-COMMUNICABLE DISEASES. PART 2: PREVALENCE OF GERMLINE VARIANTS ASSOCIATED WITH CANCERS AMONG RESIDENTS OF THE RUSSIAN FEDERATION

Vladimir S. Yudin¹, Aleksandra A. Mamchur¹, Ekaterina A. Nikolaeva¹, Mikhail V. Ivanov¹, Daria A. Kashtanova^{1✉}, Elena A. Zelenova¹, Sergey I. Mitrofanov¹, Ekaterina A. Snigir¹, Lyudmila P. Kuzmina², Anton A. Keskinov¹

¹Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical and Biological Agency, Moscow, Russia

²Izmerov Research Institute of Occupational Health, Moscow, Russia

Introduction. Cancer is a leading cause of mortality worldwide. Hereditary (germline) mutations in cancer predisposition genes significantly contribute to oncogenesis (accounting for 3–12.6% of adult cases). Identifying carriers of pathogenic/likely pathogenic (P/LP) variants is crucial for cancer prevention and its early detection. Large-scale data on the prevalence of such variants in the general population of the Russian Federation, essential for healthcare resource planning, have been extremely limited until now.

Objective. To assess the prevalence and spectrum of pathogenic and likely pathogenic germline variants in genes associated with cancer risk in a Russian population cohort.

Materials and methods. An analysis of whole-genome sequencing data from 116,794 participants (a representative sample of the adult population from 86 federal subjects of the Russian Federation) was conducted. The search for P/LP variants in cancer-associated genes was performed based on the ClinVar database. Variant annotation was carried out considering the level of evidence. Statistical analysis was performed using Python (v3.9.12).

Results. P/LP variants were identified in 26 cancer-associated genes among 2643 participants (2.26%). The most frequent variant was rs36053993 (*MUTYH*, 0.28%), with its frequency in the Russian Federation being lower than in global databases (ALFA, gnomAD). Level A variants (only those with the “reviewed by expert panel” level of evidence) were carried by 0.8% of individuals collectively. Level B variants (those with the “reviewed by expert panel” and “criteria provided, multiple submitters, no conflicts” levels of evidence) were carried by 2.08%. The inclusion of less reliable evidence levels (C, D) increased this proportion only marginally, to 2.26%. A statistically significant decrease in the proportion of carriers of Level A variants with age was observed ($p = 0.007$), while the overall proportion of P/LP variant carriers decreased only slightly ($p = 0.17$). Substantial geographical variability in prevalence was identified, ranging from 865 (Bashkortostan) to 6250 (Chechnia) variants per 100,000 population. In Moscow, the prevalence was 2340 per 100,000.

Conclusions. The study revealed a significant “hidden burden” of P/LP oncogenic germline variant carriers (2.26%) in the Russian population. The primary burden is attributed to variants with a high level of evidence (Level B). The substantial variability in prevalence across regions of the Russian Federation necessitates a differentiated approach to healthcare resource planning. The data obtained substantiate the need for implementing genetic screening programs (especially for individuals with a family history) and enhanced oncological surveillance for carriers. This represents a strategic direction for reducing cancer incidence and mortality in high genetic risk groups within the Russian Federation.

Keywords: chronic non-communicable diseases; oncological diseases; germline genetic variants; whole-genome sequencing; hereditary predisposition

For citation: Yudin V.S., Mamchur A.A., Nikolaeva E.A., Ivanov M.V., Kashtanova D.A., Zelenova E.A., Mitrofanov S.I., Snigir E.A., Kuzmina L.P., Keskinov A.A. Hidden burden of chronic non-communicable diseases. Part 2: Prevalence of germline variants associated with cancers among residents of the Russian Federation. *Extreme Medicine*. 2026;28(1):28–36. <https://doi.org/10.47183/mes.2025-366>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study was approved by the Local Ethical Committee of the Centre for Strategic Planning of the FMBA of Russia (Minutes No. 5 of 28.12.2020; Minutes No. 2 of 01.06.2021). For the creation of the Genetic Database (GDB) of the Centre for Strategic Planning of the FMBA of Russia, voluntary informed consent for participation in the study was obtained from all participants, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Potential conflict of interest: Anton A. Keskinov is the scientific editor of the *Extreme Medicine* journal. The remaining authors declare no conflict of interest.

✉ Daria A. Kashtanova DKashtanova@cspfmba.ru

Received: 4 Aug. 2025 **Revised:** 8 Oct. 2025 **Accepted:** 22 Oct. 2025 **Online first:** 28 Nov. 2025

УДК 631.523:616-006.04

СКРЫТОЕ БРЕМЯ ХРОНИЧЕСКИХ НЕИНФЕКЦИОННЫХ ЗАБОЛЕВАНИЙ. ЧАСТЬ 2: РАСПРОСТРАНЕННОСТЬ ГЕРМИНАЛЬНЫХ ВАРИАНТОВ, АССОЦИИРОВАННЫХ С ОНКОЛОГИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ, СРЕДИ ЖИТЕЛЕЙ РОССИЙСКОЙ ФЕДЕРАЦИИ

В.С. Юдин¹, А.А. Мамчур¹, Е.А. Николаева¹, М.В. Иванов¹, Д.А. Каштанова^{1✉}, Е.А. Зеленова¹, С.И. Митрофанов¹, Е.А. Снигирь¹, Л.П. Кузьмина², А.А. Кескинов¹

¹Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

²Научно-исследовательский институт медицины труда им. академика Н.Ф. Измерова, Москва, Россия

© V.S. Yudin, A.A. Mamchur, E.A. Nikolaeva, M.V. Ivanov, D.A. Kashtanova, E.A. Zelenova, S.I. Mitrofanov, E.A. Snigir, L.P. Kuzmina, A.A. Keskinov, 2025

Введение. Онкологические заболевания — ведущая причина смертности в мире. Наследственные (герминальные) мутации в генах предрасположенности к раку вносят существенный вклад в онкогенез (3–12,6% случаев у взрослых). Выявление носителей патогенных / вероятно патогенных (P/LP) вариантов критически важно для профилактики и ранней диагностики рака. Крупномасштабные данные о распространенности таких вариантов в общей популяции Российской Федерации для планирования ресурсов здравоохранения ранее были крайне ограничены.

Цель. Оценить распространенность и спектр патогенных и вероятно патогенных герминальных вариантов в генах, ассоциированных с риском развития онкологических заболеваний, в российской популяционной когорте.

Материалы и методы. Проведен анализ данных полногеномного секвенирования 116 794 участников (репрезентативная выборка взрослого населения из 86 субъектов РФ). Поиск P/LP-вариантов в онкоассоциированных генах выполнен на основе базы данных ClinVar. Аннотация вариантов проводилась с учетом уровня доказательности. Статистический анализ выполнен с использованием Python (v3.9.12).

Результаты. P/LP-варианты обнаружены в 26 онкоассоциированных генах у 2643 (2,26%) участников. Наиболее частый вариант — rs36053993 (*MUTYH*, 0,28%), частота в РФ ниже мировых баз (ALFA, gnomAD). Варианты уровня А (только варианты с уровнем доказательности «проверено экспертной комиссией») имели суммарно 0,8% людей, уровня В (варианты с уровнями доказательности «проверено экспертной комиссией» и «критерии предоставлены несколькими заявителями, конфликтов нет») — 2,08%, добавление менее надежных уровней (С, D) увеличивало показатель лишь до 2,26%. Наблюдали статистически значимое снижение доли носителей вариантов уровня А с возрастом ($p = 0,007$), общая доля носителей P/LP-вариантов снижалась незначительно ($p = 0,17$). Выявлена значительная географическая вариабельность распространенности: от 865 (Республика Башкортостан) до 6250 (Чеченская Республика) вариантов на 100 тыс. населения. В Москве — 2340 на 100 тыс.

Выводы. Исследование выявило значимое «скрытое бремя» носительства P/LP онкогерминальных вариантов (2,26%) в российской популяции. Основное бремя обусловлено вариантами с высоким уровнем доказательности (уровень В). Значительная вариабельность распространенности по регионам РФ требует дифференцированного подхода к планированию ресурсов здравоохранения. Полученные данные обосновывают необходимость внедрения программ генетического скрининга (особенно для лиц с семейным анамнезом) и усиленного онконаблюдения для носителей, что является стратегическим направлением для снижения онкозаболеваемости и смертности в группах высокого генетического риска в РФ.

Ключевые слова: хронические неинфекционные заболевания; онкологические заболевания; герминальные генетические варианты; полногеномное секвенирование; наследственная предрасположенность

Для цитирования: Юдин В.С., Мамчур А.А., Николаева Е.А., Иванов М.В., Каштанова Д.А., Зеленова Е.А., Митрофанов С.И., Снигирь Е.А., Кузьмина Л.П., Кескинов А.А. Скрытое бремя хронических неинфекционных заболеваний. Часть 2: распространенность герминальных вариантов, ассоциированных с онкологическими заболеваниями, среди жителей Российской Федерации. *Медицина экстремальных ситуаций*. 2026;28(1):28–36. <https://doi.org/10.47183/mes.2025-366>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование одобрено локальным этическим комитетом ФГБУ «ЦСП» ФМБА России (протокол № 5 от 28.12.2020, протокол № 2 от 01.06.2021). Для создания базы данных популяционных частот (GDB) ФГБУ «ЦСП» ФМБА России от всех участников получено добровольное информированное согласие на участие в исследовании, проведенном в соответствии с принципами Хельсинкской декларации.

Потенциальный конфликт интересов: А.А. Кескинов — научный редактор журнала «Медицина экстремальных ситуаций», остальные авторы заявляют об отсутствии конфликта интересов.

✉ Каштанова Дарья Андреевна DKashtanova@cspfmba.ru

Статья поступила: 06.08.2025 **После доработки:** 06.10.2025 **Принята к публикации:** 27.10.2025 **Online first:** 28.11.2025

INTRODUCTION

Cancer remains one of the most significant challenges in global public health and a leading cause of mortality worldwide [1, 2]. Combating oncological diseases requires a comprehensive approach, including understanding and accounting for their risk factors. Despite advancements in diagnostic methods and increased coverage of screening programs, malignant neoplasms are currently definitively identified once they have become clinically apparent. Therefore, developing technologies for the preventive detection and prediction of their development is critically important.

Hereditary (germline) mutations significantly contribute to oncogenesis, accounting for approximately 3–12.6% of adult cancer cases and 8.5–10% of childhood cancer cases [3, 4]. The influence of heredity on cancer risk varies from low-penetrance susceptibility (caused by common germline variants that increase relative risk by

1.5–2 times) through moderate penetrance (risk increases by 2–5 times) to high-penetrance predisposition associated with rare germline variants (risk increase more than fivefold) [5, 6]. Predispositions with moderate and high penetrance are typically inherited in an autosomal dominant pattern and are associated with mutations in Cancer Predisposition Genes (CPGs), of which over 100 have been identified [7]. Screening for such variants is particularly important in families with a significant family history of cancer [8–10].

Clinical whole-exome (WES) and whole-genome (WGS) sequencing have become key tools for identifying hereditary cancer syndromes. WES effectively and economically detects pathogenic variants in the coding regions of known CPGs and Cancer Driver Genes (CDGs) [11], rendering it the primary method for clinical practice and screening.

Whole-genome sequencing offers a unique advantage through the ability to detect pathogenic variants

across the entire genome, including non-coding regulatory regions and structural variants potentially involved in hereditary predisposition [12]. However, its application is limited by higher costs and the complexity of interpreting findings in non-coding regions.

In 2013, the American College of Medical Genetics and Genomics (ACMG) issued recommendations directing laboratories to actively seek and report to patients the identification of pathogenic variants in a specific list of 56 genes associated with pathological but potentially manageable conditions [13]. This list, which includes several CPGs (e.g., *BRCA1*, *BRCA2*, *TP53*), was revised in 2017 (ACMG v2.0, 59 genes) and further in 2021 [14]. Although the original recommendations relied on a binary variant classification (“known pathogenic” / “expected pathogenic”), a transition to the modern, five-category ACMG pathogenicity system was strongly recommended.

This approach inevitably uncovers genetic variants unrelated to the primary indication for testing but possessing independent clinical significance. Such findings, known as secondary findings (SF), may indicate a risk for developing severe diseases (including some forms of cancer) for which effective preventive measures or early-stage treatments exist [15]. Identifying these germline mutations is critical for cancer prevention and early diagnosis in at-risk groups.

Determining the true frequency of pathogenic/likely pathogenic (P/LP) variant carriers in CPGs within the general population, not just among cancer patients, is of particular interest to healthcare systems. Knowledge of such a “genetic burden” allows for the proactive planning of medical interventions, such as estimating the need for genetic counseling, genetic testing, enhanced screening programs, and preventive measures (e.g., mammography + breasts MRI for *BRCA1/2* mutation carriers, colonoscopy for Lynch syndrome). Such planning enables the assessment of the burden on the healthcare system: forecasting the demand for expensive genetic tests, specialized diagnostics, and preventive treatments on a regional or national scale, ultimately optimizing cancer prevention strategies for individuals at high risk.

Conducting large-scale population studies to assess the frequency of germline mutations presents significant challenges. These include high costs, especially when utilizing WGS, and the necessity of examining large cohorts to obtain statistically significant data, particularly for rare genetic variants and in ethnically diverse populations. A particular ongoing difficulty lies in the interpretation of the results from such studies, due to the need for accurate variant classification according to stringent criteria (e.g., ACMG/AMP (Association for Molecular Pathology)) [16]. This demands substantial expert and computational resources, especially for automation (e.g., using InterVar) [17] and for the analysis of non-coding

variants and structural variants. Furthermore, challenges entail ethical and organizational aspects: obtaining informed consent, ensuring data confidentiality, and organizing the collection of biomaterials.

Despite the challenges, research in this sphere is underway. Projects such as The Cancer Genome Atlas [17] and The Pancancer Analysis of Whole Genomes [4] have provided data on the frequency of germline variants among cancer patients (8% and 17%, respectively). Studies in the general population, primarily involving cohorts of European-American and African-American ancestry, estimate the prevalence of P/LP variants in genes from the American College of Medical Genetics and Genomics (ACMG) secondary findings list to range 0.8–7%. However, data derived from tumor tissues do not reflect the frequency in the healthy population.

As far as the Russian Federation is concerned, large-scale studies on the prevalence of germline P/LP variants in CPGs/CDGs in the population are extremely limited. Our previous study, which included data from nearly 77,000 individuals, was a significant step toward describing the genetic burden of hereditary cancer in the Russian population [19] but required expansion.

In the present study, we aim to assess the prevalence and spectrum of pathogenic and likely pathogenic germline variants in genes associated with the risk of developing oncological diseases within an expanded Russian population cohort. The results obtained are of significant importance for understanding the hidden genetic burden of hereditary cancer in the Russian Federation. This information is necessary for planning and optimizing resources within regional healthcare systems required for the prevention, early diagnosis, and management of patients from high genetic risk groups.

MATERIALS AND METHODS

Study participants and ethical considerations

This study is a continuation of a large-scale epidemiological project initiated in 2019 by the Centre for Strategic Planning of the FMBA of Russia to investigate the prevalence of genetic variants associated with the risk of chronic diseases, including oncological conditions, among the adult population across 86 federal subjects of the Russian Federation.

The study was conducted using a population-based sample from the Genetic Database (GDB) of the Centre for Strategic Planning of the FMBA of Russia.¹ The randomized representative sample comprised 116,794 individuals from 86 federal subjects of the Russian Federation. The demographic characteristics of the participants (age and sex distribution) are presented in Figure 1. The median age was 51 [22;73] years for men and 51 [23;75] years for women.

¹ Genetic Database (GDB). FMBA of Russia. Application v1.1.3 of 17.03.2025. Database v59.1 of 03.10.2024. URL: <https://gdbpop.nir.cspfmba.ru> (access date 02.08.2025) (In Russ.)

Whole-genome sequencing

DNA was extracted from whole blood samples using the MagAttract HMW DNA Kit (Qiagen, Germany) on an automated robotic station. Whole-genome sequencing libraries were prepared using the Illumina DNA Prep kit (Illumina, USA) according to the manufacturer's instructions. Whole-genome sequencing was performed on the Illumina NovaSeq 6000 platform using the S4 Reagent Kit (300 cycles) (Illumina, USA) for paired-end reads of 2×150 bp.

During the demultiplexing stage, sequencing data in BCL format were converted to FASTQ format using Illumina bcl2fastq v2.20. The sequencing run quality was controlled using Illumina Sequencing Analysis Viewer v2.4.7, while the read quality (FASTQ.GZ format) was assessed using the FastQC v0.11.9 bioinformatic tool. Alignment to the reference genome (GRCh38) was performed on the Dragen Bio-IT platform (Illumina, USA). Quality control of alignments (BAM format) was conducted using DRAGEN, FastQC v0.11.9, samtools v1.13, and mosdepth v0.3.1. All samples passed quality control based on key metrics, including the percentage of duplicated reads and the number of unaligned reads. The final mean genome coverage per sample was at least 30x. Detection of single nucleotide polymorphisms (SNPs) and short insertions/deletions (up to 50 bp) was performed using Strelka2 [9]; only variants passing the PASS filter were selected.

Search for cardiac germline variants

A search for pathogenic and likely pathogenic genetic variants associated with the development of oncological diseases was conducted within the study sample, based on the ClinVar database. The following levels of evidence for the relationship between a genetic variant and the development of the corresponding phenotype, as provided by ClinVar (in descending order of strength), were used for annotating the obtained list of variants:

- "Practice guideline"
Highest level. Interpretation is based on official clinical guidelines from authoritative professional societies. This information represents the most reliable data for clinical decision making.
- "Expert panel"
Very high level. The variant has been thoroughly evaluated and approved by an independent expert group (e.g., within the ClinGen consortium). This also constitutes an extremely reliable basis for clinical action.
- "Multiple submitters"
High reliability. Multiple laboratories have independently reached the same interpretation of the variant, and no conflicting data exist. This is a reliable indicator for use in practice.
- "Single submitter"

Moderate reliability. The interpretation originates from only one laboratory or research group. Such data require caution and verification through scientific publications or other sources.

- "No assertion provided"

Low reliability. The submitter provided information about the variant but did not offer any clinical interpretation. This information should not be used for clinical decision making.

Statistical Data Processing

Statistical data processing and result visualization were performed using the Python programming language (v3.9.12). Intergroup comparison of the number of carriers of genetic variants was conducted using the chi-square test.

RESULTS

Annotation results of variants associated with oncological diseases

In the analyzed sample, carriers of pathogenic and likely pathogenic variants were identified across 26 genes associated with various oncological diseases: *APC*, *BMPR1A*, *BRCA1*, *BRCA2*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF2*, *PALB2*, *PMS2*, *PTEN*, *RB1*, *RET*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *SMAD4*, *TMEM127*, *TP53*, *TSC1*, *TSC2*, *VHL*, *WT1*. At least one variant in these genes was detected in 2643 participants (2.26%). The number of unique variants identified was 467, with a cumulative frequency in the entire cohort of 1.15%, corresponding to 2307 variants per 100,000 population.

Variants in the *MUTYH* gene were the most frequently observed in the study sample. Specifically, the most common variant was rs36053993 (NC_000001.11:g.45331556C>T), with a frequency of 0.28%. According to the GDB, the frequency of this variant in the Russian population is statistically significantly lower than in other global projects. For

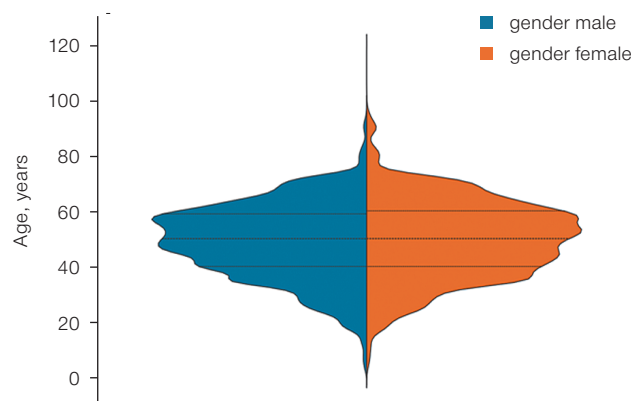


Figure prepared by the authors based on original data

Fig. 1. Age and sex distribution of the study participants

instance, its frequency in the ALFA project was 0.46% (p -value < 0.001), compared to 0.33% in gnomAD (p -value < 0.01).

The distribution of the identified variants by the level of evidence is presented in Figure 2A. Notably, none of the found variants had the “practice guideline” level of evidence. The largest number of variants (215 out of 467) had the “reviewed by expert panel” evidence level, followed by variants with the “criteria provided, multiple submitters, no conflicts” level (168 out of 467). The aforementioned most common variant, rs36053993, also belongs to this second group.

In total, Level A variants (only those with the “reviewed by expert panel” evidence level) were carried by 0.8% of individuals. Level B variants (those with “expert panel” and “multiple submitters, no conflicts” levels of evidence) were carried by 2.08%. Level C variants (those with “expert panel,” “multiple submitters, no conflicts,” and “single submitter” levels of evidence) were characteristic of 2.25% of individuals. Level D included all variants and was characteristic of 2.26% of individuals (the entire

group of carriers of pathogenic and likely pathogenic variants).

As noted above, the majority of identified variants belonged to the former two groups. Furthermore, these same groups accounted for the most prevalent variants. Consequently, Level B encompassed carriers of nearly all pathogenic and likely pathogenic variants. The inclusion of additional, less substantiated variants only marginally increased the overall carrier proportion.

It is important to note that the prevalence of pathogenic and likely pathogenic variants in a population typically exhibits an age-dependent dynamic. Carriage of these genetic variants is presumed to negatively impact survival and the likelihood of reaching old age. However, in our analysis (Fig. 2C), the proportions of P/LP carriers changed only marginally from one age group to another from a statistical standpoint (p -value = 0.17), although a trend suggesting a decrease in the carrier proportion with age was observed. This trend is more pronounced when considering only carriers of Level A variants (p -value = 0.007).

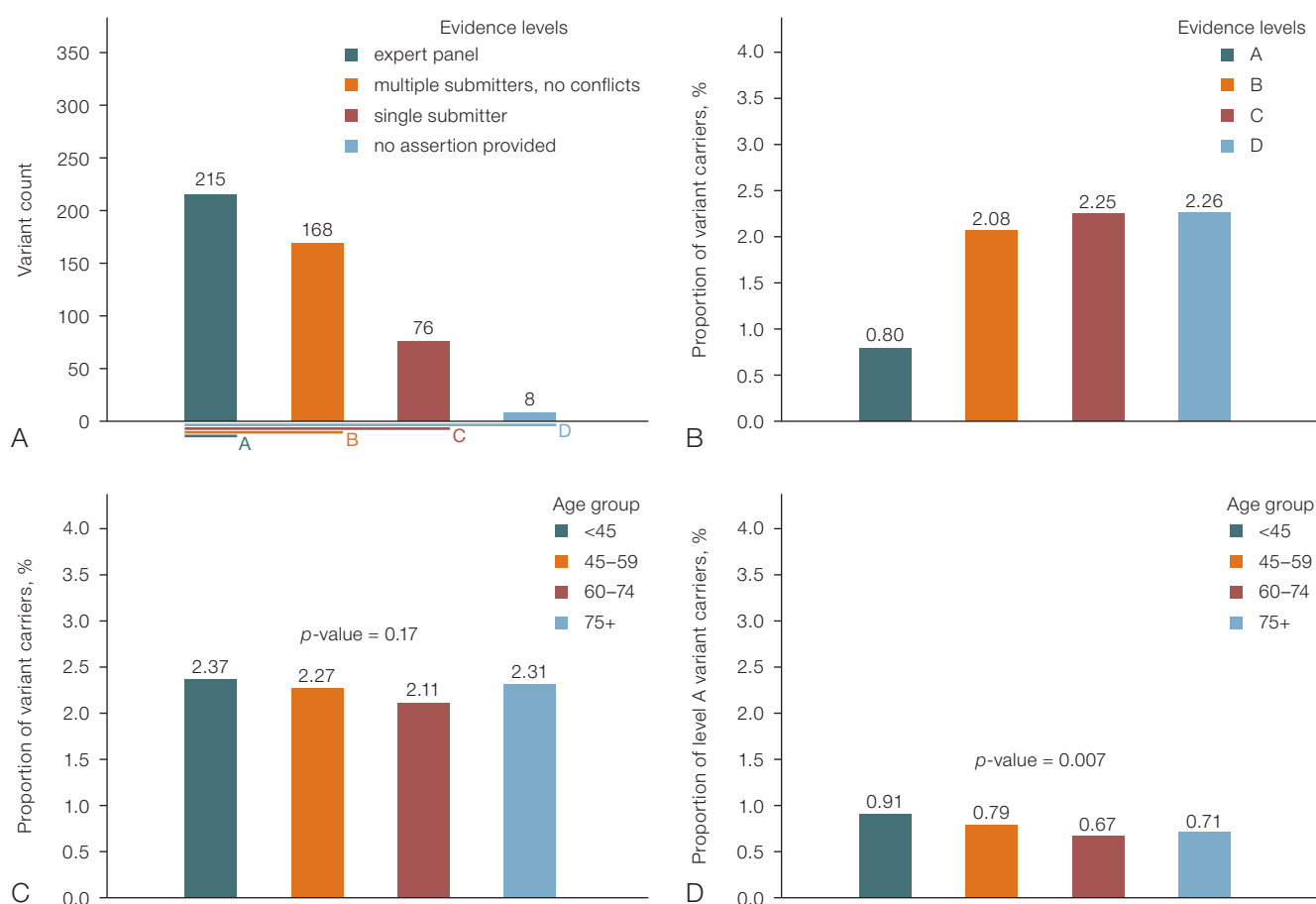


Figure prepared by the authors based on original data

Fig. 2. Annotation results of the identified genetic variants: A — frequency of variants with different evidence levels in the sample; B — proportion of carriers of variants with different evidence levels; C — proportion of carriers of all pathogenic and likely pathogenic variants associated with oncological diseases across different age groups; D — proportion of carriers of Level A variants across different age groups

Analysis of pathogenic and likely pathogenic variant distribution across Russian federal subjects

The conducted analysis also enabled the creation of a map illustrating the prevalence of pathogenic and likely pathogenic variants associated with oncological diseases across different regions of the Russian Federation (Fig. 3). The highest frequencies were observed in Chechnya (6250 variants per 100,000 population), the Republic of Komi (5484 per 100,000), Zabaykalsky Krai (4366 per 100,000), Kurgan Oblast (4219 per 100,000), and Lipetsk Oblast (3636 per 100,000). The lowest frequencies were found in the Republics of Sakha (Yakutia) (1179 per 100,000), Dagestan (1102 per 100,000), Chuvashia (1046 per 100,000), Mari El (967 per 100,000), and Bashkortostan (865 per 100,000). In Moscow, the prevalence of oncogenic germline variants was 2340 per 100,000 population, which aligns with the overall population value of 2307 mentioned earlier.

DISCUSSION

When discussing the genetics of oncological diseases, researchers and clinicians often refer to the accumulation of somatic mutations in specific cells, which stimulates the development of malignant neoplasms. However, hereditary predisposition to cancer, manifested as the carriage of pathogenic and likely pathogenic variants in cancer-associated genes, represents an equally critical risk factor. Moreover, while somatic mutations are

spontaneous and often unpredictable, germline variants are detectable from birth. Identification of oncogenic germline variants is essential for estimating disease risks at both individual and population levels, including for a specific region or an entire country.

Our large-scale study, bridging epidemiology and genetics, has identified pathogenic or likely pathogenic variants of varying evidence levels in cancer-associated genes in 2.26% of participants from a representative sample of over 116,000 residents of the Russian Federation. This sample was not enriched with individuals with established oncological diagnoses, allowing us to reveal a “hidden” genetic burden within the ostensibly healthy population. Although all identified variants exhibit incomplete penetrance and disease manifestation depends on additional factors [21], data on the prevalence of oncogenic germline variants in the population can be valuable for developing preventive care protocols. While the identified variants are characterized by incomplete penetrance, their carriage significantly increases the lifetime risk of developing specific cancer syndromes [5–8]. The observed decrease in the proportion of P/LP variant carriers with age (particularly pronounced for Level A variants with the highest level of evidence, $p = 0.007$) aligns with the concept of increased mortality or morbidity among carriers who do not survive to older age groups. This indirectly confirms the clinical significance of the identified variants and the importance of pre-symptomatic detection.

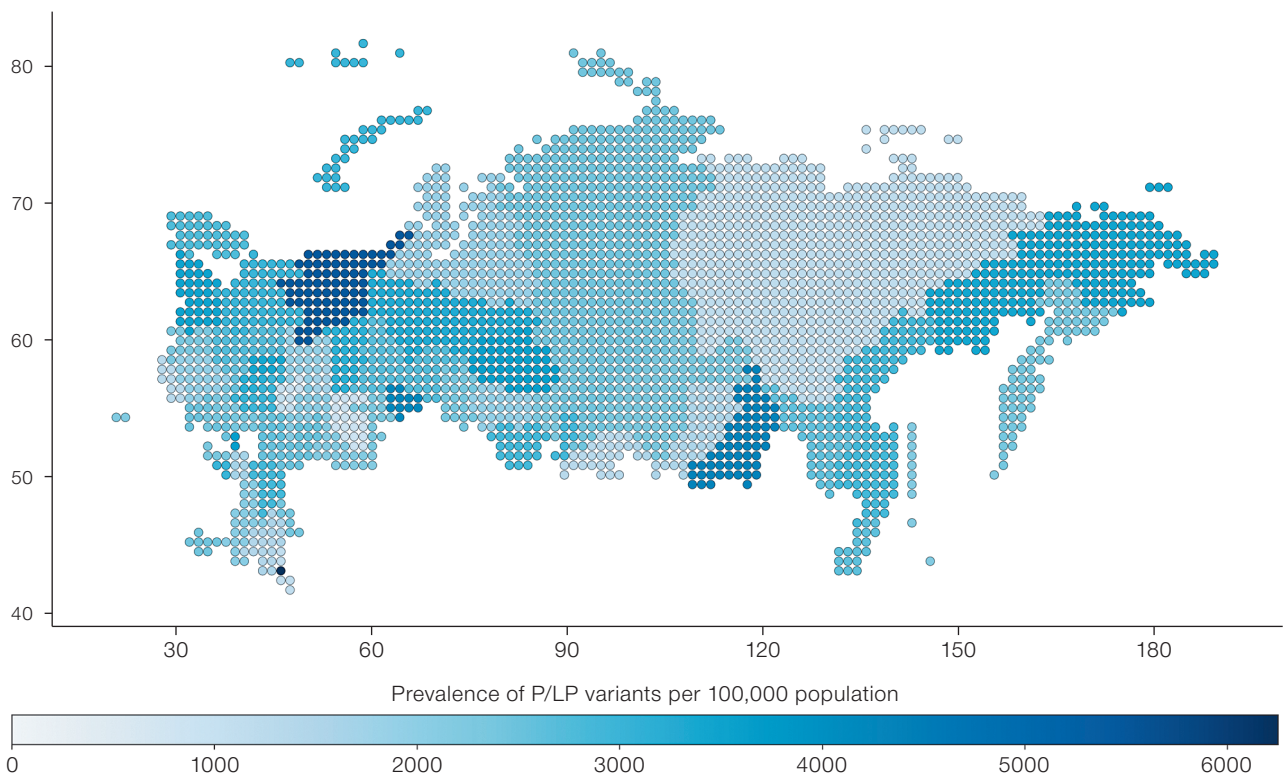


Figure prepared by the authors based on original data

Fig. 3. Prevalence map of oncogenic germline variants in the population of the Russian Federation

The use of ClinVar evidence levels enabled an assessment of the reliability of the identified associations. The fact that Level B (high and moderate evidence levels) accounts for 2.08% of carriers, while the addition of less reliable variants (Levels C and D) increases this figure only to 2.26%, holds significant practical importance. This indicates that the primary “genetic burden” is attributable to variants with a solid evidence base and also strengthens confidence in the results, justifying the prioritization of these variants when planning screening programs. The absence of variants at the “practice guideline” level underscores the need for further work on the validation and clinical interpretation of genetic findings in the Russian population.

The geographical distribution of the identified genetic variants obtained in this study is of particular value. The prevalence across different regions of Russia showed substantial variation, ranging from 865 variants per 100,000 population in Bashkortostan to 6250 variants per 100,000 in Chechnya. This disparity may be attributed to a multitude of factors. First, remote regions and isolated communities, which various ethnic groups may have constituted at different times, could be characterized by a “founder effect” or other evolutionary peculiarities.

Second, the demographic structure of a region can influence the frequency of P/LP variants. As our analysis demonstrated, the proportion of P/LP carriers decreases when moving from younger to older age groups. This is particularly significant for variants approved by an expert panel, i.e., those with the highest level of evidence. The observed trend is likely linked to the increased mortality of P/LP carriers before reaching old age, which reduces their numbers in the oldest age groups. Consequently, the prevalence of cancer-associated genetic variants will also vary depending on the demographic structure of a particular region and the predominance of younger versus older residents.

Third, the level of healthcare development in a given region is a significant factor. When cancer manifests in carriers of pathogenic/likely pathogenic variants in regions with advanced medical care, the chances of survival and achieving remission are significantly higher, thereby preserving the mutation in the population’s gene pool. It is important to consider that for some regions with small sample sizes, the frequency estimate may be less precise.

Nevertheless, knowledge of regional characteristics is critically important for differentiated planning of

healthcare system resources. The obtained data substantiate the necessity of ensuring access to confirmatory testing (especially WES/WGS), genetic screening programs (particularly for individuals with a family history), and specialized programs for enhanced cancer screening for carriers (e.g., “mammography + MRI” for *BRCA* carriers, colonoscopy for Lynch syndrome).

It is also known, for instance, that the presence of hereditary variants is significant for preventing adverse events associated with certain classical chemotherapy regimens [22], further underscoring the importance of such testing. Data on the prevalence of P/LP variants are crucial for assessing the cost-effectiveness of the widespread implementation of genetic testing and preventive programs at the population or regional level.

Thus, the early identification of carriers before the onset of disease opens the possibility for truly preventive interventions (enhanced screening, chemoprevention, prophylactic surgeries), which are capable of significantly reducing cancer incidence and mortality in this high-risk group.

CONCLUSION

The present study provides unique and large-scale data on the hidden burden of hereditary cancer predisposition in the Russian population. The identified significant carrier frequency of P/LP variants (2.26%), particularly its substantial regional variability, is of fundamental importance for understanding the genetic architecture of cancer risk in the country and serves as a powerful tool for planning and optimizing healthcare system resources.

The implementation of genetic screening programs and preventive management for carriers, based on the population data presented herein, represents a strategic direction for reducing the incidence and mortality from oncological diseases in high genetic risk groups.

Future research directions include conducting functional studies for pathogenic variants with an undetermined mechanism of action, as well as integrating genomic data with clinical and phenotypic manifestations to establish precise genotype–phenotype correlations and assess the contribution of modifying factors. Furthermore, expanding the spectrum of analyzed genes and incorporating the analysis of structural variants and copy number variations (CNVs) will provide a more comprehensive picture of hereditary predisposition.

References

1. GBD 2021 Forecasting Collaborators. Burden of disease scenarios for 204 countries and territories, 2022–2050: a forecasting analysis for the Global Burden of Disease Study 2021. *The Lancet*. 2024;403(10440):2204–56. [https://doi.org/10.1016/S0140-6736\(24\)00685-8](https://doi.org/10.1016/S0140-6736(24)00685-8)
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2024;74(3):229–63. <https://doi.org/10.3322/caac.21834>
3. Han SA, Kim SW. *BRCA* and Breast Cancer-Related High-

- Penetrance Genes. *Translational Research in Breast Cancer*. 2021;1187:473–90.
https://doi.org/10.1007/978-981-32-9620-6_25
4. Kolekar P, Balagopal V, Dong L, Liu Y, Foy S, Tran Q, et al. SJPedPanel: A Pan-Cancer Gene Panel for Childhood Malignancies to Enhance Cancer Monitoring and Early Detection. *Clinical Cancer Research*. 2024;30(18):4100–14.
<https://doi.org/10.1158/1078-0432.CCR-24-1063>
 5. Bader JS. The Panorama of Cancer Genetics. *Cancer Research*. 2021;81(10):2586–7.
<https://doi.org/10.1158/0008-5472.CAN-21-0885>
 6. Wu S, Zhu W, Thompson P, Hannun YA. Evaluating intrinsic and non-intrinsic cancer risk factors. *Nature Communications*. 2018;9(1):3490.
<https://doi.org/10.1038/s41467-018-05467-z>
 7. Hu C, Hart SN, Gnanaolivu R, Huang H, Lee KY, Na J, et al. A population-based study of genes previously implicated in breast cancer. *The New England Journal of Medicine*. 2021;384(5):440–51.
<https://doi.org/10.1056/NEJMoa2005936>
 8. Yang X, Song H, Leslie G, Engel C, Hahnen E, Auber B, et al. Ovarian and breast cancer risks associated with pathogenic variants in RAD51C and RAD51D. *Journal of National Cancer Institute*. 2020;112(12):1242–50.
<https://doi.org/10.1093/jnci/djaa030>
 9. Kastrinos F, Samadder NJ, Burt RW. Use of family history and genetic testing to determine risk of colorectal cancer. *Gastroenterology*. 2020;158(2):389–403.
<https://doi.org/10.1053/j.gastro.2019.11.029>
 10. Janssen B, Bellis S, Koller T, Tischkowitz M, Liao SS. A systematic review of predicted pathogenic *PALB2* variants: an analysis of mutational overlap between epithelial cancers. *Journal of Human Genetics*. 2020;65(2):199–205.
<https://doi.org/10.1038/s10038-019-0680-7>
 11. Wang Y, Zhou B, Ru J, Meng X, Wang Y, Liu W, et al. Advances in computational methods for identifying cancer driver genes. *Mathematical Biosciences and Engineering*. 2023;20(12):21643–69.
<https://doi.org/10.3934/mbe.2023958>
 12. Shickh S, Gutierrez Salazar M, Zakoor KR, Lazaro C, Gu J, Goltz J, et al. Exome and genome sequencing in adults with undiagnosed disease: a prospective cohort study. *Journal of Medical Genetics*. 2021;58(4):275–83.
<https://doi.org/10.1136/jmedgenet-2020-106936>
 13. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genetics in Medicine*. 2017;19(2):249–55.
<https://doi.org/10.1038/gim.2016.190>
 14. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*. 2021;23(8):1381–90.
<https://doi.org/10.1038/s41436-021-01172-3>
 15. Bowling KM, Thompson ML, Finnila CR, Hiatt SM, Latner DR, Amaral MD, et al. Genome sequencing as a first-line diagnostic test for hospitalized infants. *Genetics in Medicine*. 2022;24(4):851–61.
<https://doi.org/10.1016/j.gim.2021.11.020>
 16. Harrison SM, Biesecker LG, Rehm HL. Overview of Specifications to the ACMG/AMP Variant Interpretation Guidelines. *Current Protocols in Human Genetics*. 2019;103(1):e93.
<https://doi.org/10.1002/cphg.93>
 17. Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *ASHG*. 2017;100(2):267–80.
<https://doi.org/10.1016/j.ajhg.2017.01.004>
 18. Cancer Genome Atlas Research Network, Weinstein JN, Collison EA, Mills GB, Mills Shaw KR, Ozenberger BA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nature Genetics*. 2013;45(10):1113–20.
<https://doi.org/10.1038/ng.2764>
 19. Gusakova M, Dzhumaniiazova I, Zelenova E, Kashtanova D, Ivanov M, Mamchur A, et al. Prevalence of the cancer-associated germline variants in Russian adults and long-living individuals: using the ACMG recommendations and computational interpreters for pathogenicity assessment. *Frontiers in Oncology*. 2024;14:1420176.
<https://doi.org/10.3389/fonc.2024.1420176>
 20. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Kallberg M, et al. Strelka2: fast and accurate calling of germline and somatic variants. *Nature Methods*. 2018;15:591–4.
<https://doi.org/10.1038/s41592-018-0051-x>
 21. Virolainen SJ, VonHandorf A, Viel KCMF, Weirauch MT, Kottyan LC. Gene-environment interactions and their impact on human health. *Genes and Immunology*. 2023;24(1):1–11.
<https://doi.org/10.1038/s41435-022-00192-6>
 22. Kaehler M, Cascorbi I. Germline variants in cancer therapy. *Cancer Drug Resistance*. 2019;2(1):18–30.
<https://doi.org/10.20517/cdr.2019.05>

Authors' contributions. All authors confirm that their contributions meet the ICMJE authorship criteria. The contributions are distributed as follows: Vladimir S. Yudin — development of the research methodology and conceptual framework, formulation of scientific objectives, writing, editing, and approval of the final manuscript; Aleksandra A. Mamchur — formal data analysis, synthesis and systematization of results, data visualization, manuscript editing; Ekaterina A. Nikolaeva — synthesis of results, manuscript drafting and formatting; Mikhail V. Ivanov — software development, data administration, data visualization; Daria A. Kashtanova — scientific supervision, critical revision of the manuscript, result verification; Elena A. Zelenova — formal data analysis, conducting the study; Sergey I. Mitrofanov — software development, conducting the study; Ekaterina A. Snigir — development of methodology, conducting the study; Lyudmila P. Kuzmina — research leadership and coordination, provision of resources, result verification; Anton A. Keskinnov — project administration, provision of resources.

AUTHORS

Vladimir S. Yudin, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-9199-6258>
VYudin@cspfmmba.ru

Aleksandra A. Mamchur
<https://orcid.org/0000-0002-6025-7663>
AMamchur@cspfmmba.ru

Ekaterina A. Nikolaeva
<https://orcid.org/0000-0002-7954-2560>
Enikolaeva@cspfmmba.ru

Mikhail V. Ivanov
<https://orcid.org/0009-0004-7070-5636>
Mivanov@cspfmmba.ru

Daria A. Kashtanova, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-8977-4384>
DKashtanova@cspfmmba.ru

Elena A. Zelenova
<https://orcid.org/0000-0003-4604-7261>
Ezelenova@cspfmmba.ru

Sergey I. Mitrofanov
<https://orcid.org/0000-0003-0358-0568>
Mitrofanov@cspfmmba.ru

Ekaterina A. Snigir, Cand. Sci. (Biol.)
<https://orcid.org/0000-0003-1245-7764>
Esngir@cspfmmba.ru

Lyudmila P. Kuzmina, Dr. Sci. (Biol.)
<https://orcid.org/0000-0003-3186-8024>
lpkuzmina@mail.ru

Anton A. Keskinov, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7378-983X>
Keskinov@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-371>



ALLELIC VARIANTS OF CYP2C9, CYP2C19, CYP2D6 CYTOCHROMES IN THE RUSSIAN POPULATION: PREVALENCE AND REGIONAL DISTRIBUTION

Vladimir S. Yudin¹, Ekaterina D. Spektor^{1✉}, Aleksandra A. Mamchur¹, Mikhail V. Ivanov¹, Sergey I. Mitrofanov¹, Ludmila P. Kuzmina², Igor V. Bukhtiyarov², Anton A. Keskinov¹, Sergey M. Yudin¹, Daria A. Kashtanova¹

¹ Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical and Biological Agency, Moscow, Russia

² Izmerov Research Institute of Occupational Health, Moscow, Russia

Introduction. Determination of the *CYP2C9*, *CYP2C19*, and *CYP2D6* genotypes enables therapy personalization for a wide range of medications. The currently available diagnostic panels and international guidelines governing the scope of testing are based on global data concerning the allelic polymorphism of these genes.

Objective. To determine the distribution of allelic variants of the *CYP2C9*, *CYP2C19*, and *CYP2D6* genes in the population of the Russian Federation, taking regional characteristics into account.

Materials and methods. The study was conducted on a sample from the Genetic Database (GDB) of the Centre for Strategic Planning and Management of Biomedical Health Risks ($n = 121,442$, covering 85 Russian federal subjects). Whole-genome DNA sequencing was performed for all participants, with *CYP2C9*, *CYP2C19*, and *CYP2D6* genotyping determined using the PAnno software. In regions with a sufficient number of observations, variant frequencies and the proportions of different metabolic phenotypes were compared, identifying high-risk regions and those with an allelic structure differing from the overall population.

Results. The prevalence of impaired metabolism for *CYP2C9* substrates in the population is estimated at 33.66%, for *CYP2C19* at 25.37%, and for *CYP2D6* at 8.29%. The prevalence of accelerated metabolism for *CYP2C19* substrates is 37.37%. The following regions are identified as high-risk for the presence of impaired metabolism of substrates for at least one of the studied isoenzymes: the Republics of Chechnya, Dagestan, Ingushetia, Kabardino-Balkaria, North Ossetia, Chuvashia, Mari El, Udmurtia, Tatarstan, Tyva, Sakha (Yakutia), Kalmykia, Buryatia, Karelia, as well as Rostov Oblast, Sakhalin Oblast, Irkutsk Oblast, and Novosibirsk Oblast. This risk manifests due to both quantitative differences in the frequency of common allelic variants and the presence of rare alleles in specific regions, such as *CYP2C9**29, *CYP2C9**12, *CYP2C19**8, *CYP2D6**32, and *CYP2D6**7.

Conclusions. The obtained results provide the basis for the development of domestic diagnostic panels and for implementing a differentiated approach to pharmacogenetic testing across various biogeographic groups within the country. The introduction of such panels must be accompanied by health economic evaluation for each drug whose effectiveness and safety depends substantially on the *CYP2C9*, *CYP2C19*, and *CYP2D6* genotype.

Keywords: pharmacogenetics; precision medicine; *CYP2C9*; *CYP2C19*; *CYP2D6*

For citation: Yudin V.S., Spektor E.D., Mamchur A.A., Ivanov M.V., Mitrofanov S.I., Kuzmina L.P., Bukhtiyarov I.V., Keskinov A.A., Yudin S.M., Kashtanova D.A. Allelic variants of *CYP2C9*, *CYP2C19*, *CYP2D6* cytochromes in the Russian population: Prevalence and regional distribution. *Extreme Medicine*. 2026;28(1):37–50. <https://doi.org/10.47183/mes.2025-371>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study did not require approval from a bioethics committee, since no direct interaction with the participants was required. The study involved only secondary analysis of anonymized data. For the creation of the Genetic Database (GDB) of the Centre for Strategic Planning of the FMBA of Russia, voluntary informed consent was obtained from all participants, and the primary study was conducted in accordance with the principles of the Declaration of Helsinki.

Potential conflict of interest: Sergey M. Yudin is a member of the editorial board of *Extreme Medicine*. Anton A. Keskinov is the scientific editor of *Extreme Medicine*. The remaining co-authors declare no conflict of interest.

✉ Ekaterina D. Spektor espektor@cspfmbs.ru

Received: 5 Aug. 2025 **Revised:** 6 Oct. 2025 **Accepted:** 27 Oct. 2025 **Online first:** 17 Dec. 2025

УДК 577.2

АЛЛЕЛЬНЫЕ ВАРИАНТЫ ЦИТОХРОМОВ CYP2C9, CYP2C19, CYP2D6 У ЖИТЕЛЕЙ РОССИИ: РАСПРОСТРАНЕННОСТЬ И РЕГИОНАЛЬНОЕ РАСПРЕДЕЛЕНИЕ

В.С. Юдин¹, Е.Д. Спектор^{1✉}, А.А. Мамчур¹, М.В. Иванов¹, С.И. Митрофанов¹, Л.П. Кузьмина², И.В. Бухтияров², А.А. Кескинов¹, С.М. Юдин¹, Д.А. Каштанова¹

¹ Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

² Научно-исследовательский институт медицины труда им. академика Н.Ф. Измерова, Москва, Россия

Введение. Определение генотипа *CYP2C9*, *CYP2C19*, *CYP2D6* позволяет персонализировать терапию для широкого круга лекарственных средств. Доступные в настоящий момент диагностические панели и международные рекомендации, регламентирующие объем тестирования, базируются на общемировых данных об аллельном полиморфизме этих генов.

Цель. Определение распределения аллельных вариантов генов *CYP2C9*, *CYP2C19* и *CYP2D6* в популяции жителей РФ с учетом региональных особенностей.

© V.S. Yudin, E.D. Spektor, A.A. Mamchur, M.V. Ivanov, S.I. Mitrofanov, L.P. Kuzmina, I.V. Bukhtiyarov, A.A. Keskinov, S.M. Yudin, D.A. Kashtanova, 2025

Материалы и методы. Исследование выполнено на выборке из базы данных популяционных частот (GBD) ЦСП ФМБА России ($n = 121\,442$, охват 85 субъектов РФ). Всем участникам выполнено полногеномное секвенирование ДНК, установление генотипа *CYP2C9*, *CYP2C19* и *CYP2D6* при помощи программного обеспечения RAnno. В регионах с достаточным количеством наблюдений сопоставляли частоты вариантов и доли различных фенотипов метаболизма с выделением регионов риска и регионов с аллельной структурой, отличающейся от популяционной.

Результаты. Распространенность замедленного метаболизма субстратов *CYP2C9* в популяции оценивается в 33,66%, *CYP2C19* — в 25,37%, *CYP2D6* — в 8,29%, ускоренного метаболизма субстратов *CYP2C19* — в 37,37%. К регионам риска наличия замедленного метаболизма субстратов хотя бы одного из исследуемых изоферментов относятся Чеченская Республика, Дагестан, Ингушетия, Кабардино-Балкария, Северная Осетия, Ростовская область, Чувашия, Марий Эл, Удмуртская Республика, Татарстан, Тыва, Якутия, Калмыкия, Бурятия, Карелия, Сахалинская, Иркутская и Новосибирская области. Данный риск реализуется как за счет количественных различий во встречаемости частых аллельных вариантов, так и за счет представленности в отдельных регионах редких аллелей, таких как *CYP2C9*29*, *CYP2C9*12*, *CYP2C19*8*, *CYP2D6*32*, *CYP2D6*7*.

Выводы. Полученные результаты создают предпосылки для разработки отечественных диагностических панелей, а также для дифференцированного подхода к фармакогенетическому тестированию в разных биогеографических группах внутри страны, внедрение которых должно сопровождаться клинико-экономическим обоснованием для каждого препарата, чья эффективность или безопасность существенно зависят от генотипа *CYP2C9*, *CYP2C19* и *CYP2D6*.

Ключевые слова: фармакогенетика; персонализированная медицина; *CYP2C9*; *CYP2C19*; *CYP2D6*

Для цитирования: Юдин В.С., Спектор Е.Д., Мамчур А.А., Иванов М.В., Митрофанов С.И., Кузьмина Л.П., Бухтияров И.В., Кескинов А.А., Юдин С.М., Каштанова Д.А. Аллельные варианты цитохромов *CYP2C9*, *CYP2C19*, *CYP2D6* у жителей России: распространенность и региональное распределение. *Медицина экстремальных ситуаций*. 2026;28(1):37–50. <https://doi.org/10.47183/mes.2025-371>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование не требовало заключения биоэтического комитета, поскольку не предполагало непосредственного взаимодействия с участниками и проводилось на основе вторичного анализа деперсонифицированных данных. Для создания базы данных популяционных частот (GBD) ФГБУ «ЦСП» ФМБА России от всех участников получено добровольное информированное согласие на участие в исследовании, проведенном в соответствии с принципами Хельсинкской декларации.

Потенциальный конфликт интересов: С.М. Юдин — член редакционной коллегии журнала «Медицина экстремальных ситуаций», А.А. Кескинов — научный редактор журнала «Медицина экстремальных ситуаций», остальные авторы заявляют об отсутствии конфликта интересов.

✉ Спектор Екатерина Дмитриевна espektor@cspfmba.ru

Статья поступила: 05.08.2025 **После доработки:** 06.10.2025 **Принята к публикации:** 27.10.2025 **Online first:** 17.12.2025

INTRODUCTION

Genotyping of drug-metabolizing enzymes, a significant proportion of which are cytochrome P450 isoenzymes, plays an essential role in the personalization of pharmacotherapy. According to the studies conducted among individuals of European descent, 96.8% carry at least one genetic variant in cytochrome P450 isoenzymes capable of influencing drug metabolism [1]. The *CYP2C9*, *CYP2C19*, and *CYP2D6* isoenzymes are responsible for the metabolism of up to 45% of drugs undergoing biotransformation [2] and are classified among the so-called “very important pharmacogenes” (VIP) [3].

The *CYP2C9* cytochrome isoenzyme, which constitutes approximately 20% of all P450 isoenzymes [4], mediates the metabolism of drugs such as warfarin and other coumarin anticoagulants, phenytoin, fluvastatin, torasemide, certain sartans, oral hypoglycemic agents (primarily sulfonylurea derivatives), and non-steroidal anti-inflammatory drugs (NSAIDs), as well as a significant number of endogenous substances and non-pharmacological xenobiotics [5]. The allelic variants *CYP2C9*2* and *CYP2C9*3* are prevalent in populations of European descent, thus having been extensively studied [6]; data on allelic distribution in other populations are being accumulating on a constant basis, revealing distinct characteristics of specific biogeographic groups.

Another pharmacogenetically significant isoenzyme — *CYP2C19* — is a key enzyme in the biotransformation of clopidogrel, proton pump inhibitors, voriconazole, certain benzodiazepines, and antidepressants from the groups of selective serotonin reuptake inhibitors and tricyclic antidepressants [7]. The *CYP2C19*2* and *CYP2C19*3* variants correspond to a lack of enzyme activity, leading to a reduced rate of substrate biotransformation. For comparison, carriage of the *CYP2C19*17* allele is associated with an increased enzyme activity and a higher rate of drug metabolism [7]. In the European population, along with the wild-type *CYP2C19*1* variant, only the *2 and *17 variants are found with a relatively high frequency (>1%). The *CYP2C19*3* allele is found primarily in East Asian populations, while individuals of African descent are characterized by significant allelic diversity of this enzyme, with the presence of variants such as *9, *13, *15, and *27 [6].

Another isoenzyme — *CYP2D6* — has the broadest drug coverage and is the most polymorphic. PharmGKB lists over 180 allelic variants, a significant portion of which are either not annotated or have an annotation of unknown/uncertain function.¹ The list of drugs metabolized with its participation includes antidepressants from various pharmacological groups, antipsychotics, opioid analgesics, tamoxifen, some beta-blockers, and antiarrhythmic agents [8]. Common alleles corresponding to normal

¹ PharmGKB. URL: <https://www.pharmgkb.org>

enzyme activity, along with the wild-type *1, are *2 and *35. Alleles associated with decreased activity include *9, *17, *29, and *41, while those with absent activity include *3, *4, *5, *6, and *40. The *CYP2D6**10 haplotype occupies an intermediate position between decreased and absent activity variants [8]. Furthermore, enzyme activity can be increased due to the multiplication of alleles with normal enzyme activity (*1xN, *2xN), which leads to an accelerated rate of substrate metabolism [8, 9].

The scope of genetic variant testing is regulated by international guidelines developed by the Pharmacogenomics Working Group of the Association for Molecular Pathology (PGxWG AMP) [10–13]. These guidelines distinguish alleles recommended for testing into minimum (Tier 1) and extended (Tier 2) sets. Tier 1 includes variants with proven clinical impact and high population frequency, while Tier 2 comprises less common alleles.

Other international pharmacogenetic initiatives, such as the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Dutch Pharmacogenetics Working Group (DPWG), and the European Medicines Agency (EMA), do not prescribe specific allele sets recommended for genotyping relevant cytochrome P450 isoenzymes. Instead, they focus on establishing genotype-dosing correlations and developing clinical decision-making algorithms based on genotype (CPIC, DPWG), or on regulatory aspects, standardizing terminology, and systematizing biomarkers in pharmacogenetic studies (EMA).

The current guidelines are predominantly based on data for Western populations and fail to account for the specific characteristics of Russia, where allele distribution may differ significantly due to the unique population structure of the country. Domestic equivalents of such guidelines are currently lacking, creating a risk of underestimating clinically significant variants in patients within the Russian Federation.

This study is aimed at determining the allelic polymorphism of cytochromes *CYP2C9*, *CYP2C19*, and *CYP2D6* in the population of the Russian Federation, taking regional specificities into account.

MATERIALS AND METHODS

Study sample

The study was conducted using a population-based sample from the Genetic Database (GDB) of the Centre

for Strategic Planning of the FMBA of Russia.² The sample size comprised 121,442 individuals (57,752 (48.4%) men, mean age 50.3 ± 13.9 years; data presented as $M \pm SD$) from 85 Russian federal subjects. For the analysis of regional allele frequency distribution, participants from Moscow and Moscow Oblast, St. Petersburg and Leningrad Oblast, Sevastopol and the Crimea were pooled. The recruitment criteria for the GDB participants are described in a previously published work [14]. For comparative analysis and the formation of region-specific diagnostic panels, regions with a sample size of 250 individuals or greater were selected (72 Russian regions).

DNA extraction, sequencing, and bioinformatic analysis

Genomic DNA was extracted from whole blood samples using the MagAttract HMW DNA Kit (Qiagen, Germany) for high-molecular-weight DNA isolation with magnetic particles on automated robotic workstations. Whole-genome sequencing libraries were prepared using the Illumina DNA Prep reagent kit according to the manufacturer's instructions (Document # 1000000025416 v10, Illumina, USA) with IDT-ILMN Nextera DNA UD Indexes Set A and Set B, followed by quality control. Whole-genome sequencing was performed on the Illumina NovaSeq 6000 platform using the S4 Reagent Kit (300 cycles) (Illumina, USA) for 2×150 bp paired-end reads.

During the demultiplexing stage, sequencing data in BCL format were converted to FASTQ format using Illumina bcl2fastq2 v2.20.0.422.³ Quality control of the sequencing run was performed using Illumina Sequencing Analysis Viewer v2.4.7,⁴ while read quality (FASTQ.GZ format) was assessed using the bioinformatic tool FastQC v0.11.9.⁵ Reads were aligned to the GRCh38.d1.vd1 reference genome⁶ using the Illumina DRAGEN Bio-IT Platform v07.021.510.3.5.7.⁷ Quality control of alignments (BAM format) was performed using DRAGEN, FastQC v0.11.9,⁸ samtools v1.13 [15], and mosdepth v0.3.1 [16]. All samples passed quality control based on key metrics. The final mean genome coverage per sample was at least 30x. Detection of small genetic variants (up to 50 bp) was performed using Illumina Strelka2 v2.9.10 [17]. The sample set was checked for duplicates using Picard CrosscheckFingerprints [18] with the built-in map file. The bioinformatic processing pipeline used was validated (F -score = 99.83%) against

² Genetic Database (GDB). FMBA of Russia. Application v1.1.3 of 17.03.2025. Database v59.1 of 03.10.2024. URL: <https://gdbpop.nir.cspfmba.ru> (access date 02.08.2025) (In Russ.).

³ bcl2fastq and bcl2fastq2 Conversion Software Downloads [Electronic resource]. URL: https://emea.support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software/downloads.html

⁴ Sequencing Analysis Viewer Support [Electronic resource]. URL: https://support.illumina.com/sequencing/sequencing_software/sequencing_analysis_viewer_sav.html

⁵ Babraham Bioinformatics — FastQC A Quality Control tool for High Throughput Sequence Data [Electronic resource]. URL: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

⁶ GDC Reference Files | NCI Genomic Data Commons [Electronic resource]. URL: <https://gdc.cancer.gov/about-data/gdc-data-processing/gdc-reference-files>

⁷ DRAGEN secondary analysis | Software for NGS data analysis [Electronic resource]. URL: <https://www.illumina.com/products/by-type/informatics-products/dragen-secondary-analysis.html>

⁸ Babraham Bioinformatics — FastQC A Quality Control tool for High Throughput Sequence Data [Electronic resource]. URL: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

the *HG001* reference genome provided by the Genome In A Bottle (GIAB) consortium (v.3.3.2) [19].

The presence of single nucleotide variants was determined from the VCF files generated during the small variant calling stage. Genotyping of *CYP2C9*, *CYP2C19*, and *CYP2D6* was performed using the PAnno software [20].

Data analysis

Data processing and visualization were performed using the Python programming language v3.9, utilizing the pandas, numpy, matplotlib, seaborn, and scipy libraries, as well as the R programming language v4.4.1, with the dplyr and ggplot2 libraries.

Allele frequency was calculated as the number of target alleles divided by twice the number of observations. The frequency of heterozygous and homozygous carriage was determined as the ratio of the corresponding genotypes to the total number of observations. Variants with an allele frequency of 1% or more were considered significant for potential inclusion in the minimum diagnostic panel (Tier 1), while those with a frequency of 0.5% or more were considered for the extended panel (Tier 2).

To compare the allele frequencies identified in the study population with global data, the standardized system of biogeographical groups developed by PharmGKB and the Bustamante Lab [21] was used. This system comprises nine groups defined by global autosomal genetic structure: African/Afro-Caribbean, American, Central/South Asian, East Asian, European, Latino, Middle Eastern, Oceanian, and Sub-Saharan African. The data sources for this system are the “1000 Genomes” and “Human Genome Diversity Project” initiatives.

Based on the allele annotations provided in PharmGKB [3], an activity score (AS) for each of the *CYP2C9*, *CYP2C19*, and *CYP2D6* isoenzymes was calculated for every sample in the cohort. The metabolic phenotype (e.g., normal, intermediate, poor; for *CYP2C19* also rapid and ultrarapid) was assigned based on the AS according to established algorithms [22–25]. For analytical convenience, intermediate and poor metabolizers were combined into a group of “impaired metabolism”, while rapid and ultrarapid metabolizers (for *CYP2C19*) were combined into a group of “accelerated metabolism”.

For each region with a sample size of 250 individuals or greater, the proportion of impaired metabolizers for *CYP2C9*, *CYP2C19*, and *CYP2D6* substrates was calculated, along with the proportion of accelerated metabolizers (for *CYP2C19* only). Proportion estimates were accompanied by a 95% confidence interval (CI) calculated using the Wilson method.

Regions at risk were identified via interval estimation, specifically those where the carrier frequency of genotypes associated with altered metabolism of the studied enzymes exceeded the population level. Subsequently,

using a z-test with correction for multiple comparisons (Benjamini-Hochberg procedure, FDR controlled at 5%), the frequencies of functionally significant alleles in these at-risk regions were compared against the overall population data.

RESULTS

General population characteristics and allele frequencies

The distribution of allele frequencies and carrier frequencies in the studied population sample for variants with a prevalence exceeding 0.5% are presented in Table 1.

For the *CYP2C9* gene, the *2 and *3 variants, which occur with a frequency greater than 1%, appear to be prospective for inclusion in the minimum testing panel. The *CYP2C9**29 allele could potentially be included in the extended testing panel recommended for the Russian population, as its occurrence exceeds 0.5% and its carrier frequency is over 1%.

Among *CYP2C19** allelic variants that exceed the 1% prevalence threshold, along with the wild-type *1, are the *17, *2, and *38 alleles. However, *CYP2C19**38 corresponds to normal enzyme activity, similar to the wild-type, therefore testing for its carriage is not justified. Thus, it is reasonable to include *CYP2C19**2 and *CYP2C19**17 in the minimum testing panel for *CYP2C19** in the Russian Federation, while placing *CYP2C19**3, which has a prevalence of less than 1%, into the extended diagnostic panel.

The *CYP2D6** gene is characterized by significant allelic diversity. Among the variants frequent in the Russian population (*2, *3, *4, *9, *10, *33, *35, *41), those with a confirmed impact on enzyme activity are *3 (AS = 0), *4 (AS = 0), *9 (AS = 0.5), *10 (AS = 0.5), and *41 (AS = 0.5). These alleles are candidates for inclusion in the minimum diagnostic panel. Among the less common variants, but with a frequency exceeding 0.5%, those affecting the final enzyme activity include *CYP2D6**6 (AS = 0) and *CYP2D6**59 (AS = 0.5).

On the basis of frequency and functional criteria, minimum and extended diagnostic panels for genotyping the *CYP2C9*, *CYP2C19*, and *CYP2D6* isoenzymes, relevant for the residents of the Russian Federation, are formulated (Fig. 1, Table 2).

Regional features of allele frequency distribution

Given that the genetic structures of individual Russian regions can vary significantly, this nationwide panel should be modified where necessary. Regions where the *CYP2C9* allele distribution implied a different optimal diagnostic panel are highlighted in Figure 1A. The corresponding information for *CYP2C19* and *CYP2D6* is presented in Figure 1B and Table 2, respectively.

Most differences were characterized by the inclusion of the *29 allele in the minimum panel due to its

Table 1. Prevalence rates of *CYP2C9*, *CYP2C19*, and *CYP2D6* allelic variants in the general population with allele frequency > 0.5%

Allelic variant	Allele frequency, %	Carrier frequency, %	Heterozygous carrier frequency in the sample, %	Homozygous carrier frequency in the sample, %	PGxWG AMP diagnostic guidelines
CYP2C9					
*1	81.31	96.38	30.13	66.24	Tier 1: *2, *3, *5, *6, *8, *11 Tier 2: *12, *13, *15
*2	9.59	18.2	17.23	0.97	
*3	7.76	14.82	14.11	0.7	
*29	0.98	1.91	1.88	0.04	
CYP2C19					
*1	52.99	77.63	49.11	28.52	Tier 1: *2, *3, *17 Tier 2: *4, *5, *6, *7, *8, *9, *10, *35
*17	25.55	44.19	37.2	6.99	
*2	12.81	23.73	21.96	1.77	
*38	7.5	14.36	13.69	0.67	
*3	0.58	1.14	1.11	0.03	
CYP2D6					
*1	37.86	60.06	44.35	15.7	Tier 1: *2, *3, *4, *5, *6, *9, *10, *17, *29, *41 Tier 2: *7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56, *59
*2	18.13	32.06	27.84	4.22	
*4	15.29	27.48	24.42	3.06	
*35	8.98	16.64	15.32	1.32	
*41	7.37	13.87	12.99	0.88	
*10	3.68	6.5	5.64	0.86	
*33	1.91	3.71	3.6	0.11	
*9	1.51	2.94	2.86	0.08	
*3	1.12	2.19	2.14	0.06	
*6	0.9	1.76	1.73	0.04	
*39	0.85	1.58	1.45	0.13	
*59	0.68	1.32	1.28	0.04	

Table compiled by the authors based on original data

Note: rows are sorted in descending order of allele frequency; alleles with a frequency of less than 1% are highlighted in green color.

frequency exceeding the 1% threshold. Furthermore, in the Republics of Chuvashia (6.32%) and Mari El (5.61%), its frequency surpassed the 5% threshold. However, some qualitative differences were also noted: the presence of the *CYP2C9**11 variant in the residents of the Republic of Komi, and the *CYP2C9**12 variant in the residents of the Republics of Dagestan and Chechnya.

For residents of a significant number of Russian regions, encompassing almost the entire Central and Northwestern Federal Districts, many regions of the North Caucasian District, and to a lesser extent other macro-regions, testing for the *CYP2C19**3 allele is not

relevant, even within the extended panel, as its frequency does not exceed the 0.5% threshold. Conversely, in the Republics of Sakha (Yakutia), Buryatia, Mari El, Bashkortostan, Kalmykia, and Tyva, this variant is sufficiently prevalent to recommend testing for its carriage as part of the minimum diagnostic panel. In terms of qualitative distinctions, the Yamalo-Nenets Autonomous Okrug and Zabaykalsky Krai stand out, where the *CYP2C19**8 allele (AS = 0) is present with frequencies of 0.51% and 0.58%, respectively.

Due to the high allelic diversity of *CYP2D6* and the presence of several alleles in the population structure with a prevalence of around 1%, the observed variety

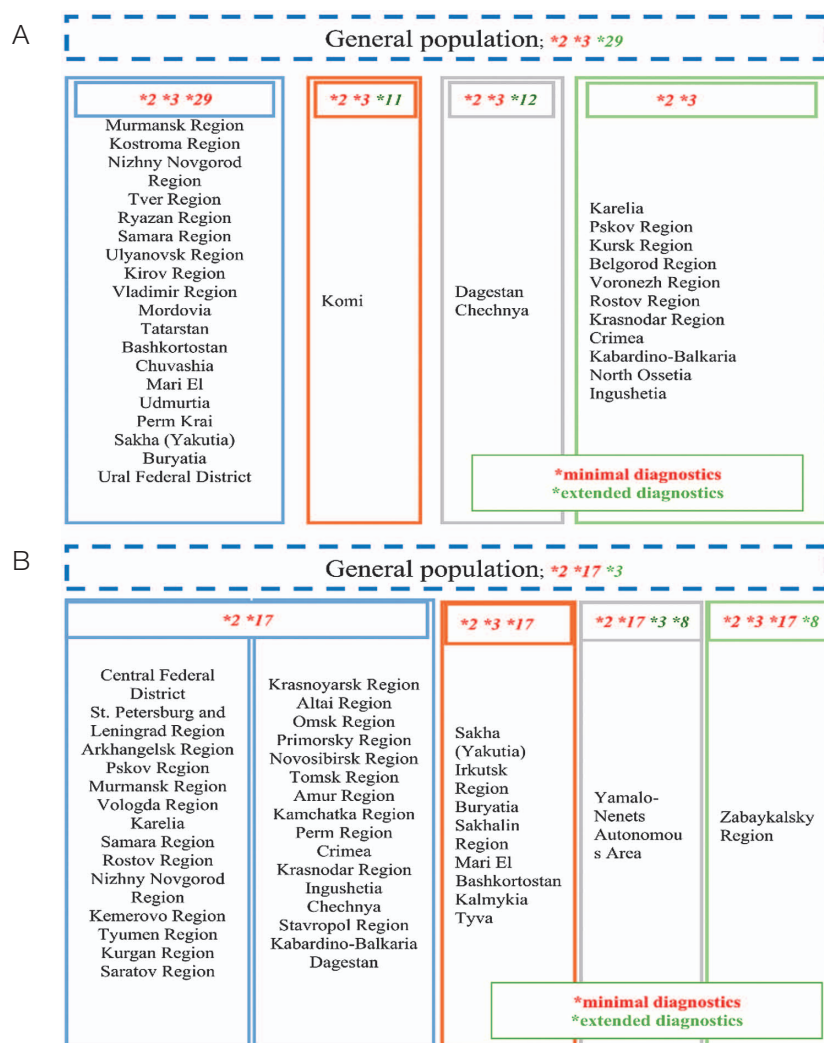


Figure prepared by the authors based on original data

Fig. 1. Diagnostic panels for *CYP2C9* and *CYP2C19* genotyping in residents of the Russian Federation across specific regions: A — *CYP2C9* allelic variants; regional groups and their corresponding optimal diagnostic panels are highlighted in blocks; B — *CYP2C19* allelic variants; regional groups and their corresponding optimal diagnostic panels are highlighted in blocks; Ural Federal District (UFD) includes the Kurgan, Sverdlovsk, Tyumen, and Chelyabinsk Oblasts, as well as the Khanty-Mansi Autonomous Okrug and Yamalo-Nenets Autonomous Okrug; LR — Leningrad Oblast; the Central Federal District (CFD), excluding the Oryol, Tambov, Lipetsk, and Bryansk Oblasts, which were not included in the analysis due to a low number of observations

of diagnostic panel configurations is expected for combinatorial reasons. The *4, *10, and *41 alleles were consistently present in the minimum diagnostic panel across all studied groups, making them the most significant for *CYP2D6* genotyping in the Russian population. The *3, *6, *9, and *59 alleles were typically included in one of the panels. Conversely, some regions (North Ossetia, Adygea, Sakha (Yakutia), Tyva, Kalmykia, and to a lesser extent Buryatia) were characterized by lower genetic diversity for this enzyme. Therefore, the use of less extensive diagnostic panels in these regions appears justified.

Qualitative differences include the presence of the *32 (AS = 0.25) and *7 (AS = 0) alleles in certain subpopulations. The *CYP2D6**32 allele was found in the Republics of Mari El (0.85%), Dagestan (0.75%), Kabardino-Balkaria

(0.79%), Chuvashia (0.67%), and Chechnya (1.43%), as well as in Kostroma Oblast (0.52%). A significant presence of the *CYP2D6**7 allele was identified in Ingushetia (1.39%) and Chechnya (1.64%).

Several of the studied samples also contained rare alleles *74 and *117 with unknown function; on this basis, they were not included in the diagnostic panels. *CYP2D6**74 was found in Khabarovsk (0.53%), Penza (0.6%) and Kaluga (0.52%) Oblasts, as well as in Zabaykalsky Krai (0.8%) and Kamchatka (0.69%). In turn, carriage of *CYP2D6**117 with a frequency exceeding 0.5% was recorded in some northern regions (Arkhangelsk Oblast — 0.58%, Vologda Oblast — 0.56%, Karelia — 0.87%, Murmansk Oblast — 0.53%), as well as in Tyumen Oblast (0.58%) and Perm Oblast (0.68%).

Table 2. Diagnostic panels for *CYP2D6* genotyping in residents of specific regions of the Russian Federation

Region	Minimum diagnostic panel of allelic variants	Extended diagnostic panel
General population	*3, *4, *9, *10, *41	*6, *59
Kurgan Oblast	*3, *4, *10, *41	*6, *9, *59
Tula Oblast Vladimir Oblast Saratov Oblast	*3, *4, *6, *9, *10, *41, *59	–
Karelia Bashkortostan	*3, *4, *9, *10, *41, *59	*6
Smolensk Oblast	*3, *4, *9, *10, *41	*59
Mordovia	*3, *4, *6, *9, *10, *41	–
Stavropol Oblast Nizhny Novgorod Oblast Belgorod Oblast Kaluga Oblast Penza Oblast Chelyabinsk Oblast Kemerovo Oblast Arkhangelsk Oblast Ivanovo Oblast Tver Oblast Perm Oblast Altai Oblast Irkutsk Oblast Zabaykalsky Krai Khabarovsk Oblast Khanty-Mansi Autonomous Okrug	*3, *4, *6, *9, *10, *41	*59
Yaroslavl Oblast Amur Oblast	*4, *6, *9, *10, *41	*3
Mari El	*4, *10, *41	*3, *6, *9, *32
Kostroma Oblast	*3, *4, *9, *10, *41, *59	*6, *32
Murmansk Oblast	*3, *4, *10, *41	*6, *9
Dagestan Kabardino-Balkaria	*4, *10, *41	*6, *9, *32
North Ossetia — Alania Adygea	*4, *10, *41	*6, *9
Ingushetia	*4, *7, *10, *41	–
Chuvashia	*4, *10, *41	*6, *9, *32, *59
Chechnya	*4, *7, *10, *32, *41	–
Tyva	*4, *10, *41	–
Sakha (Yakutia) Kalmykia	*4, *10, *41	*3, *9
Buryatia	*4, *10, *41	*3, *6, *9
Komi	*3, *4, *6, *9, *10, *41	–
Krasnodar Oblast	*4, *6, *10, *41	*3, *59
Crimea Samara Oblast Orenburg Oblast Udmurtia Sakhalin Oblast	*4, *9, *10, *41	*3, *6, *59

Table 2 (continued)

Region	Minimum diagnostic panel of allelic variants	Extended diagnostic panel
Tatarstan Kursk Oblast Kamchatka Oblast	*4, *9, *10, *41	*3, *6
Novosibirsk Oblast Tyumen Oblast	*4, *6, *9, *10, *41	*3, *59

Table compiled by the authors based on original data

Note: “–” no data available

Comparison with global data

A comparison of the allele frequencies of cytochromes CYP2C9, CYP2C19, and CYP2D6 identified in the study population with global data (Fig. 2) demonstrates that the frequency distribution of alleles for these enzymes in the Russian population sample is similar to that in the general European population. The *CYP2C19**38 allele was not considered due to being classified as a sub-allele of the wild-type *CYP2C19**1.001 in earlier nomenclatures [26] and, therefore, equated to

*CYP2C19**1 in the global resources used for allele frequency comparison.

The presence of the *CYP2C9**29 allele (approximately 1%) in the Russian population sample is the most notable distinction. Indeed, it is poorly represented in the European population (0.03%) and absent in other biogeographical groups except for the East Asian group, where its prevalence is also low (0.22%). The *CYP2D6**10 allele occurred significantly more frequently in the Russian population compared to the European population (3.7% vs. 1.57%, $p = 0.000$), which is likely

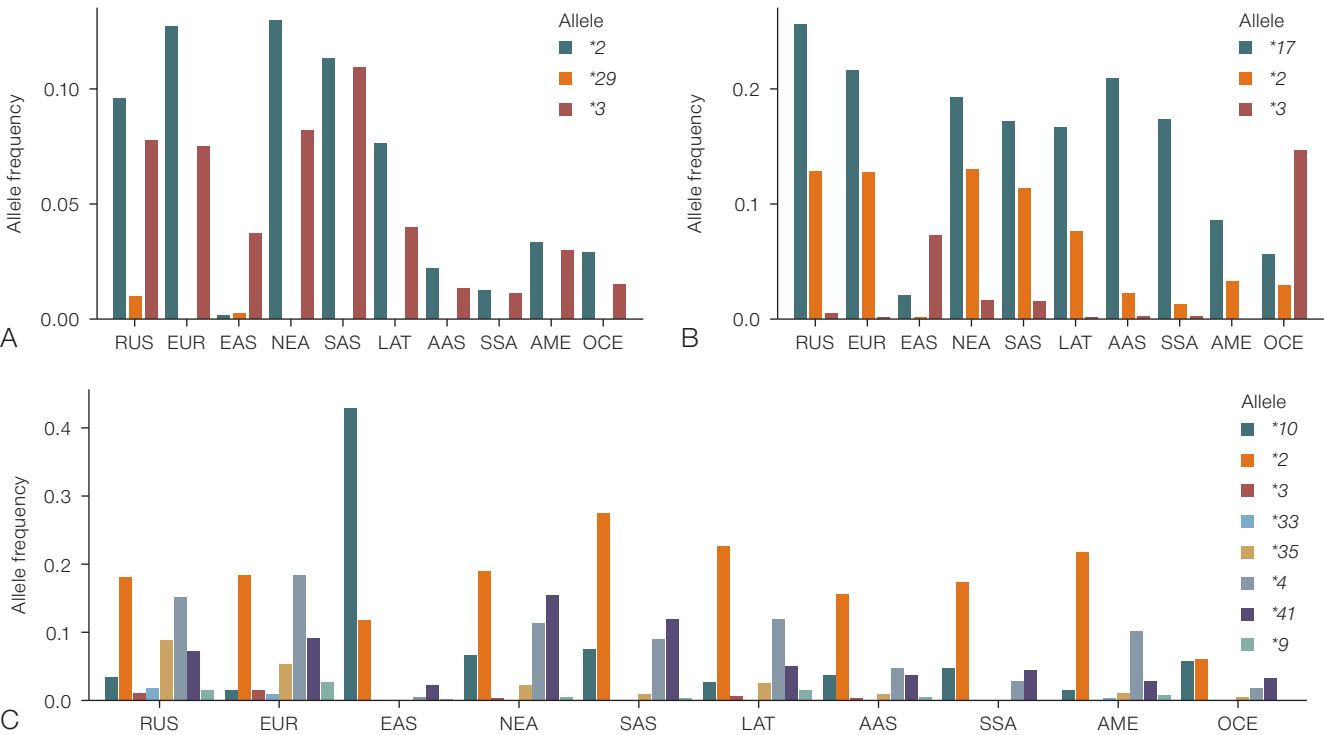


Figure prepared by the authors based on original data

Fig. 2. Distribution of *CYP2C9* (A), *CYP2C19* (B), and *CYP2D6* (C) isoenzyme alleles with a frequency > 1% in the Russian population across major biogeographical groups: allele frequencies (Y-axis) are presented as absolute values; the X-axis represents populations: RUS — Russian (sample from the present study); EUR — European and US residents of European ancestry; EAS — East Asian (China, Japan, Korea, partially Southeast Asia); NEA — Middle Eastern (Middle East, Caucasus, North Africa); SAS — South and Central Asian (Pakistan, India, Bangladesh); LAT — Latino, groups with mixed Indigenous American, European, and African ancestry; AAC — Afro-Caribbean; SSA — Sub-Saharan African; AME — Indigenous peoples of North and South America; OCE — Indigenous populations of Australia, Oceania, and New Guinea

attributable to influences from Middle Eastern (6.77%) and East Asian (42.84%) populations.

Metabolic phenotypes of CYP2C9, CYP2C19, and CYP2D6 substrates: population-wide assessment and identification of high-risk regions

In the general population, the *CYP2C9* genotype corresponding to normal metabolism of its substrates ($AS = 2$) was found with a frequency of 66.34%, intermediate metabolism ($1 \leq AS < 2$) — 31.35%, and poor metabolism ($AS < 1$) — 2.31%. Thus, the total proportion of individuals with reduced *CYP2C9* enzyme activity was 33.66%, or approximately one third of the population. The regional distribution of the proportion of individuals with reduced *CYP2C9* enzyme activity is presented in Figure 4.

According to the obtained interval estimates, six federal subjects can be identified where the frequency of genotypes associated with reduced metabolism of *CYP2C9* substrates is statistically significantly higher than the general population average. These high-risk regions are the Republics of Chechnya, Dagestan, Ingushetia, Kabardino-Balkaria, North Ossetia, Chuvashia, Mari El, Udmurtia, Tatarstan, Chuvashia, Mari El,

Udmurtia, and Tatarstan. Conversely, Buryatia, Kalmykia, Tyva, and Sakha (Yakutia) seem favorable from this perspective. To clarify the pattern of allele distribution in the risk regions, a comparison of *CYP2C9* variant frequencies with general population estimates was conducted (Fig. 3B, 3C).

The obtained results demonstrated sufficient heterogeneity among the identified high-risk regions regarding the contribution of specific allelic variants to the increased likelihood of carrying genotypes for impaired *CYP2C9* substrate metabolism. This risk was driven by an increased carrier frequency of the *CYP2C9**3 allele in the Republics of Chechnya (18.89%, $p\text{-adj} = 9.4 \times 10^{-27}$), Kabardino-Balkaria (12.68%, $p\text{-adj} = 3.8 \times 10^{-13}$), Dagestan (17.69%, $p\text{-adj} = 1.7 \times 10^{-89}$), Ingushetia (18.28%, $p\text{-adj} = 3.1 \times 10^{-40}$), and North Ossetia (11.04%, $p\text{-adj} = 9.3 \times 10^{-5}$). Additionally, Chechnya and Dagestan showed a somewhat higher representation of the *CYP2C9**12 allele (0.71%, $p\text{-adj} = 2.1 \times 10^{-19}$ and 0.04%, $p\text{-adj} = 8.3 \times 10^{-22}$, respectively). Conversely, the *CYP2C9**29 allele was relatively highly prevalent in the Republics of Mari El (5.61%, $p\text{-adj} = 1.1 \times 10^{-43}$), Chuvashia (6.32%, $p\text{-adj} = 3.3 \times 10^{-104}$), Tatarstan (2.15%, $p\text{-adj} = 2.5 \times 10^{-5}$), and Udmurtia (2.17%, $p\text{-adj} = 0.009$),

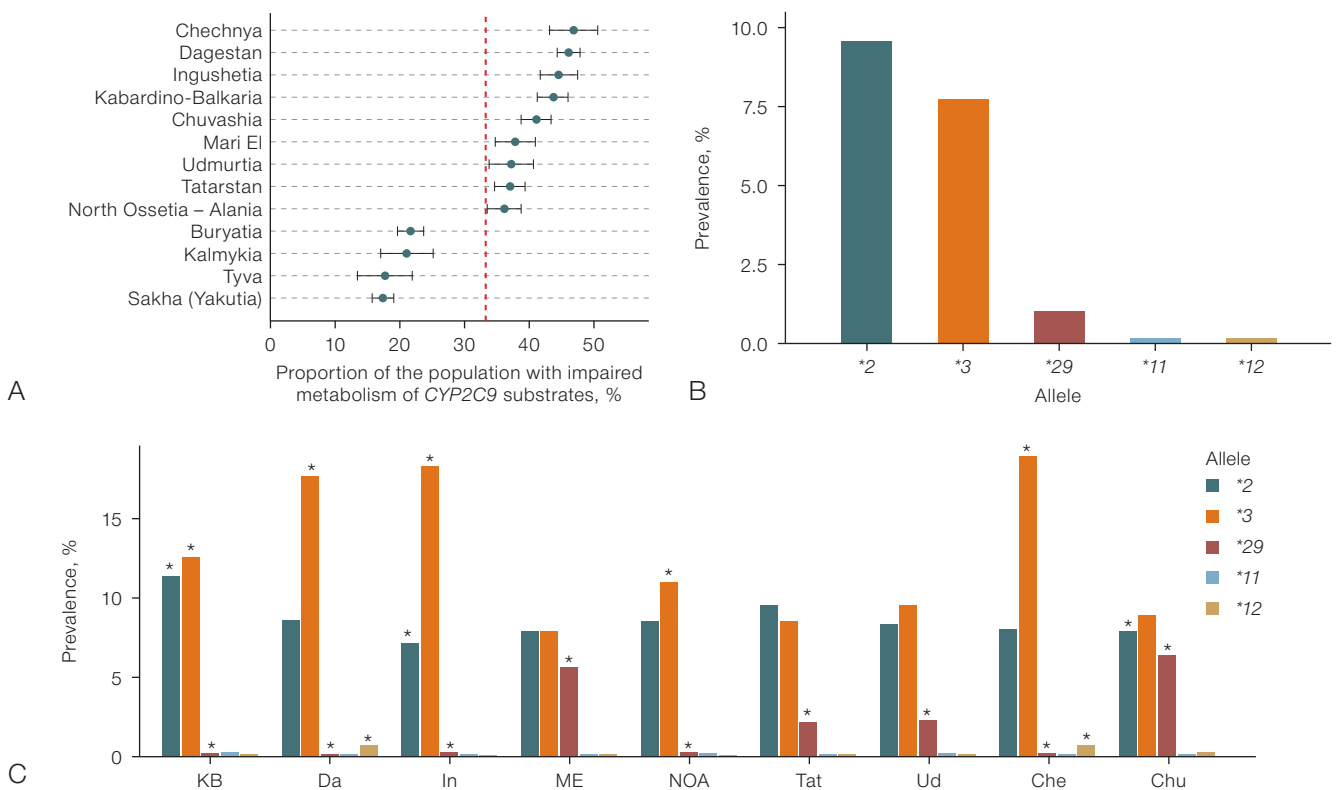


Figure prepared by the authors based on original data

Fig. 3. Population characteristics of *CYP2C9* allelic polymorphism: A — Regional distribution of the proportion of carriers of *CYP2C9* genotypes corresponding to slow metabolism of enzyme substrates (regions with a proportion differing from the population are presented); B — population allelic distribution of *CYP2C9*; C — allelic distribution of *CYP2C9* in regions with increased frequency of slow/intermediate metabolism genotypes compared to the population

Note: the red dashed line represents the population estimate; KB — Kabardino-Balkaria; Da — Dagestan; In — Ingushetia; ME — Mari El; NOA — North Ossetia — Alania; Tat — Tatarstan; Ud — Udmurtia; Che — Chechnya; Chu — Chuvash; “*” — frequencies statistically significantly different from the population values ($p\text{-adj} < 0.05$).

while the prevalence of the *2 and *3 alleles in these regions was comparable to the general population.

In the sample under study, the majority of individuals had a normal (37.26%) or rapid (30.38%) metabolic phenotype for CYP2C19 substrates. The proportion of carriers of intermediate metabolism genotypes was 23.26%, ultrarapid (*17/*17) — 6.99%, and poor — 2.11%. The combined proportions of carriers of impaired (intermediate + poor) and accelerated (rapid + ultrarapid) metabolism genotypes were 25.37% and 37.37%, respectively. Regional variations in the proportions of accelerated and impaired metabolizers are presented in Figure 4A.

Tyva, Yakutia (Sakha), Kalmykia, and Buryatia were characterized by a substantial decrease in the proportion

of individuals with the accelerated metabolism phenotype and a simultaneous significant increase in the percentage of impaired metabolizers. The latter also holds true, although to a lesser extent, for Sakhalin Oblast, Irkutsk Oblast, Novosibirsk Oblast, and Karelia. Regions such as Ingushetia, Chechnya, Kabardino-Balkaria, and Dagestan had lower proportions of both accelerated and impaired metabolizers of CYP2C19 substrates compared to the population averages. An increased representation of the rapid/ultrarapid metabolic phenotype was observed among residents of Chuvashia and Altai, Smolensk, Moscow, Rostov, Tver, Leningrad, Tula, Kemerovo, Voronezh, Ivanovo, Arkhangelsk, and Pskov Oblasts.

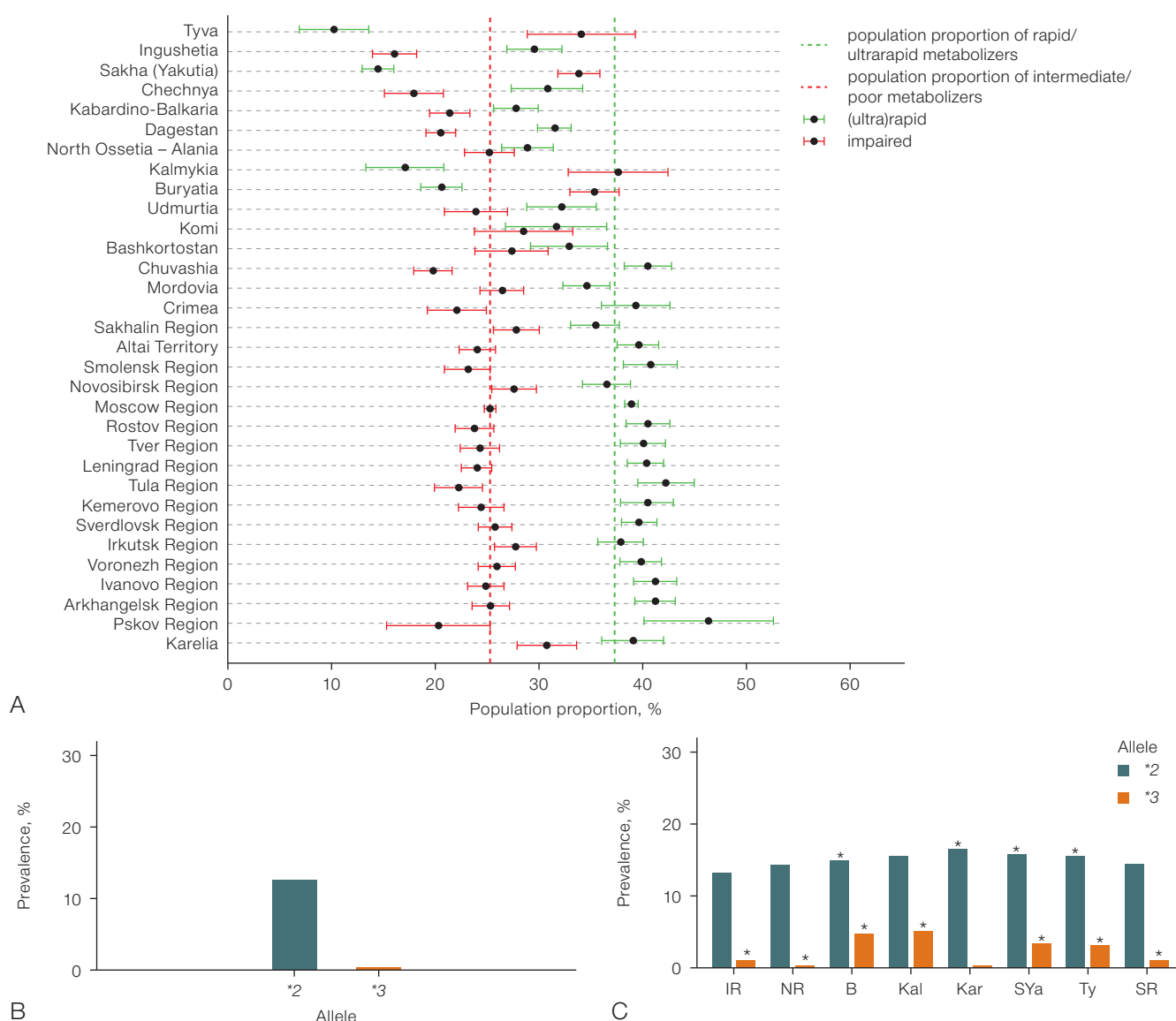


Figure prepared by the authors based on original data

Fig. 4. Population characteristics of CYP2C19 allelic polymorphism: A — regional distribution of CYP2C19 metabolic phenotypes (regions with a proportion differing from the population are presented); B — population allele distribution of CYP2C19; C — allelic distribution of CYP2C19 in regions with increased frequency of slow/intermediate metabolism genotypes compared to the population

Note: Ty — Tyva; SYa — Sakha (Yakutia); Kal — Kalmykia; B — Buryatia; Kar — Karelia; SR — Sakhalin Oblast; NR — Novosibirsk Oblast; IR — Irkutsk Oblast; “*” — frequencies statistically significantly different from the population values ($p\text{-adj} < 0.05$).

Variations in the proportion of carriers of the rapid or ultrarapid metabolism phenotype can only be attributed to changes in the prevalence of the *17 allele, as being the sole allele associated with increased CYP2C19 enzyme activity. For regions with an increased prevalence of the impaired metabolism phenotype (Tyva, Yakutia (Sakha), Kalmykia, Buryatia, Karelia, Sakhalin, Novosibirsk, and Irkutsk Oblasts), the nature of the allelic distribution was detailed in comparison with the population average (Fig. 4B, 4C).

The obtained results of allelic variant distribution in regions at risk for impaired metabolism of CYP2C19 substrates indicate that this risk is realized not through the presence of rare alleles in these geographic groups, but due to a higher representation of slow alleles that are also prevalent in the general population, namely *CYP2C19*3* and, to a lesser extent, *CYP2C19*2*.

The population sample under study was characterized by a normal metabolism of CYP2D6 substrates in 91.72% of cases. The intermediate metabolism phenotype was observed in 4.32% of instances, and the poor metabolism phenotype in 3.97%. The overall proportion of impaired metabolism in the population was 8.29%; the corresponding data are presented in Figure 5A.

Residents of Tyva, Yakutia (Sakha), Kalmykia, Chuvashia, Udmurtia, Buryatia, Mari El, Bashkortostan, and Arkhangelsk Oblast had a low risk of carrying a genotype corresponding to impaired metabolism of the enzyme substrates. A somewhat higher proportion of intermediate/poor metabolizers was recorded in Rostov Oblast (9.84%), Dagestan (10.17%), and Kabardino-Balkaria (10.05%). The allele distribution for CYP2D6 in these regions is presented in Figures 5B and 5C.

When comparing the frequency of individual alleles in the high-risk regions with the general population estimates, it was found that Kabardino-Balkaria has a higher frequency of *CYP2D6*41* (10.46%, $p\text{-adj} = 1.3 \times 10^{-5}$) and *CYP2D6*10* (4.92%, $p\text{-adj} = 0.022$). In Dagestan, a higher frequency of the *CYP2D6*2* (20.96%, $p\text{-adj} = 1.5 \times 10^{-4}$) and *CYP2D6*4* (17.98%, $p\text{-adj} = 1.5 \times 10^{-4}$) alleles is observed. In Rostov Oblast, after correction for multiple comparisons, no significant differences in the frequency of major alleles were detected.

DISCUSSION

The results of our study, obtained from a large and representative population sample, contribute to the

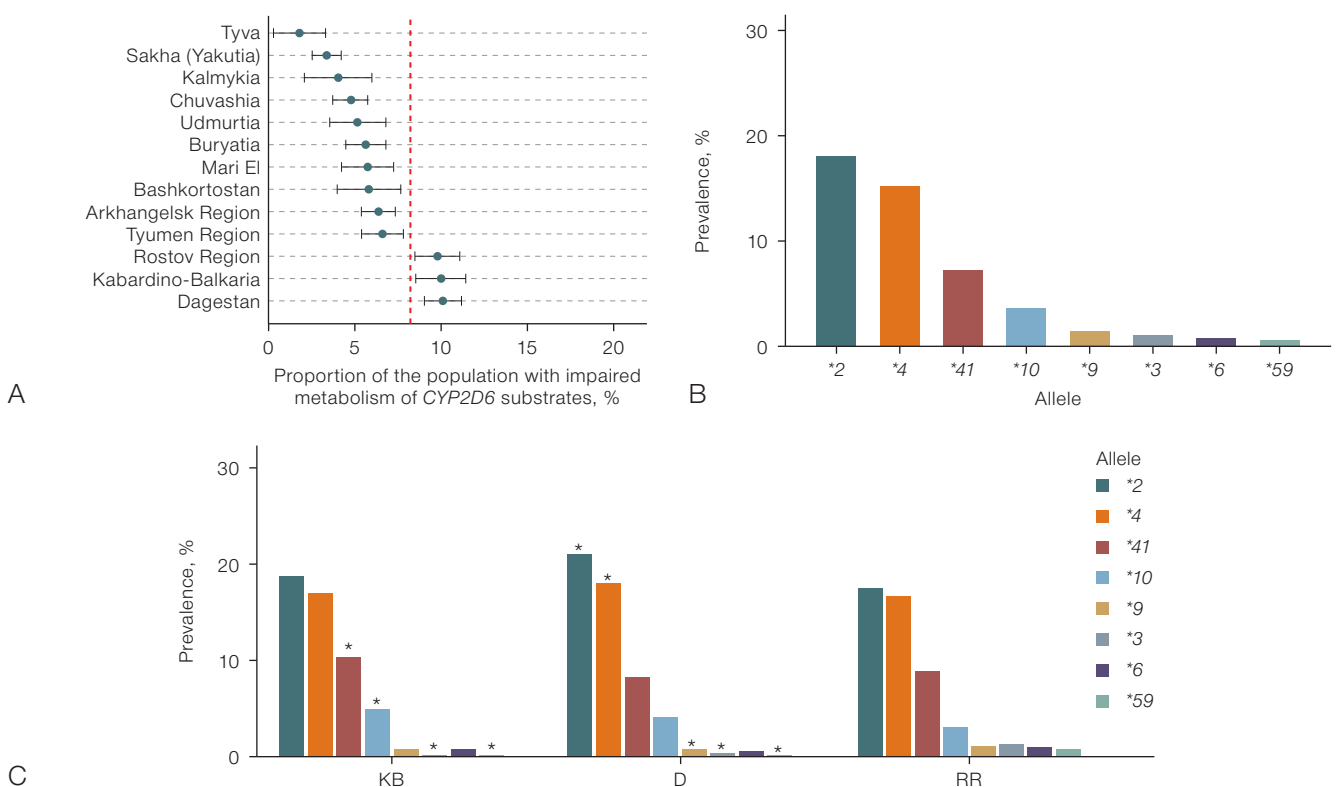


Figure prepared by the authors based on original data

Fig. 5. Allele distribution for CYP2D6 in Rostov Region, Dagestan, and Kabardino-Balkaria: A — regional distribution of the proportion of CYP2D6 genotype carriers corresponding to slow metabolism of enzyme substrates (regions with a proportion differing from the population are presented); B — population-level allelic distribution of CYP2D6; C — allelic distribution of CYP2D6 in regions with elevated carrier frequency of slow/intermediate metabolism genotypes compared to the population baseline

Note: RR — Rostov Oblast; KB — Kabardino-Balkaria; D — Dagestan; “*” — frequencies statistically significantly different from the population values ($p\text{-adj} < 0.05$).

understanding of *CYP2C9*, *CYP2C19*, and *CYP2D6* allelic polymorphism in the Russian population and provide a basis for specifying the scope of pharmacogenetic testing at the regional level. The widespread implementation of such testing, which is an integral part of personalized medicine, is impossible without the creation of accessible diagnostic tools. These tools, in turn, are limited by a predefined set of variants they can detect. Our results demonstrate that the panels formulated in the AMP guidelines are not optimal for use in Russian clinical practice.

The minimum allele testing panel for *CYP2C9* proposed by AMP covers haplotypes *2, *3, *5, *6, *8, and *11, while the extended panel includes *12, *13, and *15 [11]. However, in the Russian population, all these variants, except for *2 and *3, have a frequency of less than 0.5%, making testing for their carriage impractical. The AMP guidelines are primarily oriented toward residents of the United States and Europe. The inclusion of most of these alleles in these recommendations is likely due to their relatively high prevalence in individuals of African ancestry [6]. In contrast, *CYP2C9**29, which is found in approximately 2% of the Russian population and is associated with reduced *CYP2C9* enzyme activity (AS = 0.5), is not included even in the extended AMP recommendation panel.

The minimum *CYP2C19* genotyping panel proposed by AMP includes haplotypes *2, *3, and *17 [12]. However, according to our data, *CYP2C19**3 warrants inclusion in the minimum diagnostic panel only in specific regions, not for the general population, as its prevalence is less than 1%. Due to the lack of a functional role, this variant was not included in our proposed diagnostic panels for *CYP2C19*. The alleles suggested by AMP for extended diagnostics (*4, *5, *6, *7, *8, *9, *10, *35) do not exceed the 0.5% prevalence threshold in the Russian population. Therefore, the rationale for their inclusion in extended domestic diagnostic panels appears questionable.

As far as *CYP2D6* genotyping recommendations are concerned, the common ground is the set of alleles *2, *3, *4, *9, *10, and *41. Haplotypes *5, *6, *17, and *29 are not relevant for the Russian population, yet they are included in the AMP minimum panel [10], likely due to their high frequency in African and Latino populations. Potential candidates for inclusion in the extended diagnostic panel for the Russian population are the *6 (AS = 0) and *59 (AS = 0.5) alleles, as they are not exceptionally rare and are characterized by an impact on enzyme activity. Among these, only the *CYP2D6**59 allele is present in the extended list proposed by AMP, while over ten other alleles irrelevant for residents of Russia are listed.

Regional differences in the allele structure reflect the genetic heterogeneity of the Russian population. In the population of Komi, the *CYP2C9**11 variant (AS = 0.5) met the criteria for inclusion in the extended diagnostic panel (prevalence 0.71%). According to global data, this

variant is found only in individuals of Sub-Saharan African ancestry [21] and is included in the AMP minimum panel. A similar situation is observed for the *CYP2C9**12 variant (AS = 0.5), registered in residents of Dagestan (0.75%) and Chechnya (0.72%). This variant is not found in global populations except for the admixed Middle Eastern population, where it has an extremely low prevalence (less than 0.1%) [21].

Among the *CYP2C19* haplotypes that are extremely rare in global populations, we identified the *8 variant (AS = 0) in residents of the Yamalo-Nenets Autonomous Okrug (0.51%) and Zabaykalsky Krai (0.58%). Notably, this allele is not detected at all in individuals of East Asian ancestry, which might be expected given the geography of these regions, and its inclusion is not recommended by AMP even in the extended panel. The *CYP2D6**32, *CYP2D6**74, and *CYP2D6**117 haplotypes, present in specific regions of the Russian Federation, are not included in the AMP diagnostic panels. *CYP2D6**32 is characterized by reduced enzyme activity (AS = 0.25) and is exceptionally rare, found only in the general European (0.3%), Central/South Asian (0.2%), and Southern African (0.04%) populations.⁹ However, its prevalence in Chechnya exceeds 1%, and in the Republics of Mari El, Chuvashia, Dagestan, and Kabardino-Balkaria, it ranges 0.5–1%.

The functional role of *CYP2D6**74 and *CYP2D6**117 remains undefined. However, according to CPIC guidelines, carriers of these variants are classified as intermediate metabolizers [22, 27]. We did not include these variants in the recommended diagnostic panels due to insufficiently robust evidence supporting their functional significance; however, this position regarding the necessity of their inclusion may be revised as more data accumulates. *CYP2D6**74, which occurs with a frequency of 0.5–0.6% in Khabarovsk Oblast, Zabaykalsky Krai, Kamchatka, Penza Oblast, and Kaluga Oblast, is not documented in any biogeographical group except for individuals of African ancestry. Conversely, *CYP2D6**117 is observed exclusively in Europeans with a frequency of less than 1%.¹⁰

The largest Russian biotech companies manufacturing reagent components for polymerase chain reaction include primers for detecting *CYP2C9**2 and *CYP2C9**3 carriage in their product lines. Consequently, accounting for the *CYP2C9**29 allele, which is relevant for the Russian population, and to a lesser extent *CYP2C9**12, is currently only feasible through whole-genome sequencing and not by routine diagnostic methods. For *CYP2C19*, the commonly available testing panel (*CYP2C19**2, *CYP2C19**3, *CYP2C19**17) covers the necessary variants for most regions, with the exception of the Yamalo-Nenets Autonomous Okrug and Zabaykalsky Krai, where testing for *CYP2C19**8 carriage holds relative relevance as part of extended diagnostics. Given the high variability of the *CYP2D6* gene, the required diagnostic scope is also quite extensive, encompassing the detection of alleles *3, *4,

⁹ PharmGKB. URL: <https://www.pharmgkb.org>

¹⁰ Ibid.

*10, *41, *6, and *9. Furthermore, private laboratories also perform testing for gene deletion (*5) and duplication. The optimal testing panel for the Russian population should also include the *59 allele, and in specific regions, the *32 and *7 alleles.

An important limitation of our study is the analysis of only single nucleotide polymorphisms and haplotypes predicted based on their basis. Structural variants, such as large deletions (e.g., *CYP2D6**5) or gene copy number variations (e.g., *CYP2D6**xN), were not considered due to the inability to accurately determine gene copy number or other major structural variations from short-read whole-genome sequencing data with an average coverage of 30–40x. This may have led to an underestimation of the frequencies of some clinically significant alleles where structural variants contribute substantially to the metabolic phenotype. According to the literature, *CYP2D6**xN is prevalent in East Asian populations [6]; therefore, a similar pattern might be expected in the eastern regions of Russia.

Furthermore, it should be noted that the proposed regional panels serve as an important basis for personalizing pharmacotherapy. However, their implementation into clinical practice requires prior health-economic evaluation. It is crucial to assess the balance between the costs of genetic testing and the potential harm from failing to account for functionally significant variants. For drugs with a narrow therapeutic index, pre-emptive genotyping may be economically justified provided it has a favorable tolerability profile and entails no serious

threats; conversely, it might be considered excessive when the drug proves ineffective. Therefore, when developing clinical guidelines, not only population genetic data must be considered, but also the severity and risks of the preventable adverse drug reactions, cost parameters, and clinical outcomes for specific medications.

CONCLUSIONS

In general, the Russian population shares similarities with the European population regarding the allelic polymorphism of *CYP2C9*, *CYP2C19*, and *CYP2D6*. However, there are distinctive features both at the general population level and within specific regions, demonstrating significant population heterogeneity. This underscores the importance of not only developing domestic diagnostic panels but also adopting a differentiated approach to pharmacogenetic testing across various biogeographic groups within the country.

The presence of alleles considered exceptionally rare by global standards — such as *CYP2C9**29, *CYP2C9**11, *CYP2C9**12, *CYP2C19**8, *CYP2D6**32, *CYP2D6**7, *CYP2D6**74, and *CYP2D6**117 — has been established within the population structure or in specific biogeographic groups of the Russian Federation.

The implementation of the proposed diagnostic panels into clinical practice must be accompanied by health-economic evaluation for each drug whose effectiveness and safety depends significantly on the *CYP2C9*, *CYP2C19*, and *CYP2D6* genotype.

References

- Dawes M, Aloise MN, Ang S, Cullis P, Dawes D, Fraser R, et al. Introducing pharmacogenetic testing with clinical decision support into primary care: a feasibility study. *CMAJ Open*. 2016;4(3):E528–34. <https://doi.org/10.9778/cmajo.20150070>
- Baltzer Houliand M, Hansen L, Iversen E, Rasmussen HB, Larsen JB, Jorgensen S, et al. Pharmacogenetic testing of *CYP2D6*, *CYP2C19* and *CYP2C9* in Denmark: Agreement between publicly funded genotyping tests and the subsequent phenotype classification. *Basic and Clinical Pharmacology and Toxicology*. 2024;134(5):756–63. <https://doi.org/10.1111/bcpt.13990>
- Barbarino JM, Whirl-Carillo M, Altman RB, Klein TE. PharmGKB: A worldwide resource for pharmacogenomic information. *WIREs*. 2018;10(4):e1417. <https://doi.org/10.1002/wsbm.1417>
- Zhang H-F, Wang H-H, Gao N, Wei JY, Tian X, Zhao Y, et al. Physiological Content and Intrinsic Activities of 10 Cytochrome P450 Isoforms in Human Normal Liver Microsomes. *Journal of Pharmacology and Experimental Therapeutics*. 2016;358(1):83–93. <https://doi.org/10.1124/jpet.116.233635>
- Daly AK, Rettie AE, Fowler DM, Miners JO. Pharmacogenomics of *CYP2C9*: Functional and Clinical Considerations. *Journal of Personalized Medicine*. 2017;8(1):1. <https://doi.org/10.3390/jpm8010001>
- Zhou Y, Ingelman-Sundberg M, Lauschke VM. Worldwide Distribution of Cytochrome P450 Alleles: A Meta-analysis of Population-scale Sequencing Projects. *Clinical Pharmacology and Therapeutics*. 2017;102(4):688–700. <https://doi.org/10.1002/cpt.690>
- Shubbar Q, Alchakee A, Issa KW, Adi AJ, Shorbagi AI, Saber-Ayad M. From genes to drugs: *CYP2C19* and pharmacogenetics in clinical practice. *Frontiers in Pharmacology*. 2024;15:1326776. <https://doi.org/10.3389/fphar.2024.1326776>
- Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kattman BL, Malheiro AJ, ed. *Medical Genetics Summaries*. Bethesda (MD): National Center for Biotechnology Information (US);2012.
- Gong L, Klein CJ, Caudle KE, Moyer AM, Scott SA, Whirl-Carillo M, et al. Integrating Pharmacogenomics into the Broader Construct of Genomic Medicine: Efforts by the ClinGen Pharmacogenomics Working Group (PGxWG). *Clinical Chemistry*. 2025;71(1):36–44. <https://doi.org/10.1093/clinchem/hvae181>
- Pratt VM, Cavallari LH, Del Tredici AL, Hachad H, Ji Y, Moyer AM, et al. Recommendations for Clinical *CYP2C9* Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists. *Journal of Molecular Diagnostics*. 2019;21(5):746–55. <https://doi.org/10.1016/j.jmoldx.2019.04.003>
- Pratt VM, Del Tredici AL, Hachad H, Ji Y, Kalman LV, Scott SA, et al. Recommendations for Clinical *CYP2C19* Genotyping Allele Selection: A Report of the Association for Molecular Pathology. *Journal of Molecular Diagnostics*. 2018;20(3):269–76. <https://doi.org/10.1016/j.jmoldx.2018.01.011>
- Pratt VM, Cavallari LH, Fulmer ML, Gaedigk A, Hachad H, Ji Y, et al. *CYP3A4* and *CYP3A5* Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society

- for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase. *Journal of Molecular Diagnostics*. 2023;25(9):619–29.
<https://doi.org/10.1016/j.jmoldx.2023.06.008>
13. Gusakova M, Dzhumaniazova I, Zelenova E, Kashtanova D, Ivanov M, Mamchur A, et al. Prevalence of the cancer-associated germline variants in Russian adults and long-living individuals: using the ACMG recommendations and computational interpreters for pathogenicity assessment. *Frontiers in Oncology*. 2024;14:1420176.
<https://doi.org/10.3389/fonc.2024.1420176>
 14. Li H, Handsaker B, Wysoker A, Fennel T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–9.
<https://doi.org/10.1093/bioinformatics/btp352>
 15. Pedersen BS, Quinlan AR. Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics*. 2018;34(5):867–8.
<https://doi.org/10.1093/bioinformatics/btx699>
 16. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Kallberg M, et al. Strelka2: fast and accurate calling of germline and somatic variants. *Nature Methods*. 2018;15(8):591–4.
<https://doi.org/10.1038/s41592-018-0051-x>
 17. Najafov J, Najafov A. CrossCheck: an open-source web tool for high-throughput screen data analysis. *Scientific Reports*. 2017;7(1):5855.
<https://doi.org/10.1038/s41598-017-05960-3>
 18. Krusche P, Trigg L, Boutros PC, Mason CE, De La Vega FM, Moore BL, et al. Best practices for benchmarking germline small-variant calls in human genomes. *Nature Biotechnology*. 2019;37(5):555–60.
<https://doi.org/10.1038/s41587-019-0054-x>
 19. Liu Y, Lin Z, Chen Q, Chen Q, Sang L, Wang Y, et al. PAnno: A pharmacogenomics annotation tool for clinical genomic testing. *Frontiers in Pharmacology*. 2023;14:1008330.
<https://doi.org/10.3389/fphar.2023.1008330>
 20. Huddart R, Fohner AE, Whirl-Carillo M, Wojcik GL, Gignoux CR, Popejoy AB, et al. Standardized Biogeographic Grouping System for Annotating Populations in Pharmacogenetic Research. *Clinical Pharmacology and Therapeutics*. 2019;105(5):1256–62.
<https://doi.org/10.1002/cpt.1322>
 21. Karnes JH, Rettie AE, Somogyi AA, Huddart R, Fohner AE, Formea CM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C9 and HLA-B Genotypes and Phenytoin Dosing: 2020 Update. *Clinical Pharmacology and Therapeutics*. 2021;109(2):302–9.
<https://doi.org/10.1002/cpt.2008>
 22. Bousman CA, Stevenson JM, Ramsey LB, Sangkuhl K, Hicks JK, Strawn JR, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants. *Clinical Pharmacology and Therapeutics*. 2023;114(1):51–68.
<https://doi.org/10.1002/cpt.2903>
 23. Hicks JK, Sangkuhl K, Swen JJ, Ellingrod VL, Muller DJ, Shimoda K, et al. Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. *Clinical Pharmacology and Therapeutics*. 2017;102(1):37–44.
<https://doi.org/10.1002/cpt.597>
 24. Lee CR, Luzum JA, Sangkuhl K, Gammal RS, Sabatine MS, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. *Clinical Pharmacology and Therapeutics*. 2022;112(5):959–67.
<https://doi.org/10.1002/cpt.2526>
 25. Botton MR, Whirl-Carillo M, Del Tredici AL, Sangkuhl K, Cavallari LH, Agundez JAG, et al. PharmVar GeneFocus: CYP2C19. *Clinical Pharmacology and Therapeutics*. 2021;109(2):352–66.
<https://doi.org/10.1002/cpt.1973>
 26. Bousman CA, Stevenson JM, Ramsey LB, Sangkuhl K, Hicks JK, Strawn JR, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants. *Clinical Pharmacology and Therapeutics*. 2023;114(1):51–68.
<https://doi.org/10.1002/cpt.2903>

Authors' contributions. All authors confirm that their contributions meet the ICMJE authorship criteria. The largest contribution was distributed as follows: Vladimir S. Yudin — conceptualization; Ekaterina D. Spektor — methodology, manuscript creation; Aleksandra A. Mamchur — visualization; Mikhail V. Ivanov — formal analysis; Sergey I. Mitrofanov — software; Ludmila P. Kuzmina — resources; Igor V. Bukhtiyarov — research; Anton A. Keskinov — project administration; Sergey M. Yudin — research management; Daria A. Kashtanova — manuscript editing.

AUTHORS

Vladimir S. Yudin, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-9199-6258>
VYudin@cspfmmba.ru

Ekaterina D. Spektor, Cand. Sci. (Med.)
<https://orcid.org/0000-0003-0714-9476>
ESpektor@cspfmmba.ru

Aleksandra A. Mamchur
<https://orcid.org/0000-0002-6025-7663>
AMamchur@cspfmmba.ru

Mikhail V. Ivanov
<https://orcid.org/0009-0004-7070-5636>
MIvanov@cspfmmba.ru

Sergey I. Mitrofanov, Cand. Sci. (Biol.)
<https://orcid.org/0000-0003-0358-0568>
Mitrofanov@cspfmmba.ru

Ludmila P. Kuzmina, Dr. Sci. (Med.), Professor
LKuzmina@irioh.ru

Igor V. Bukhtiyarov, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-8317-2718>
Bukhtiyarov@irioh.ru

Anton A. Keskinov, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7378-983X>
Keskinov@cspfmmba.ru

Sergey M. Yudin, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-7942-8004>
info@cspfmmba.ru

Daria A. Kashtanova, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-8977-4384>
DKashtanova@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-364>



PHARMACOGENETIC FACTORS DETERMINING THE METABOLISM AND SAFETY OF AROMATIC ANTICONVULSANTS IN THE RESIDENTS OF RUSSIA

Ekaterina D. Spektor[✉], Vladimir S. Yudin, Aleksandra A. Mamchur, Antonina M. Rummyantseva, Mikhail V. Ivanov, Sergey I. Mitrofanov, Ekaterina A. Snigir, Anton A. Keskinov, Sergey M. Yudin, Daria A. Kashtanova

Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical Biological Agency of Russia, Moscow, Russia

Introduction. The use of aromatic anticonvulsants (carbamazepine, oxcarbazepine, lamotrigine, and phenytoin) is associated with the risk of severe hypersensitivity reactions, which are partly dependent on *HLA-B* and *HLA-A* genotypes (for carbamazepine/oxcarbazepine). Phenytoin and fosphenytoin are metabolized by *CYP2C9*; therefore, in the setting of genetically determined impaired drug tolerability, the likelihood of adverse events increases. Consideration of the *CYP2C9* genotype is important for developing personalized drug dosing schemes and improving treatment outcomes.

Objective. Assessment of the prevalence of major pharmacogenetic variants associated with response to aromatic anticonvulsants, with geographic stratification and identification of at-risk populations warranting preemptive genotyping prior to treatment initiation.

Materials and methods. The study was performed using samples from the Population Frequency Database (GDB) of the Federal Medical and Biological Agency (FMBA) of Russia ($n = 120,979$, covering 82 RF subjects). Whole-genome sequencing of DNA samples was conducted followed by an analysis of the carrier frequency of *HLA-B*15:02*, *HLA-B*15:11*, *HLA-A*31:01*, and various allelic variants of *CYP2C9* with calculation of the enzyme activity score. These metrics were compared across different Russian regions, identifying high-risk biogeographic groups.

Results. The *HLA-B*15:02* variant showed a prevalence of less than 1% in all regions of the Russian Federation. A relatively high carrier frequency of *HLA-B*15:11* was observed in the Republics of Buryatia and Tyva (1.3%, $p = 7.7 \times 10^{-5}$ and 3.46%, $p = 2.4 \times 10^{-3}$, respectively, compared to a population frequency of 0.11%). The elevated frequencies of *HLA-A*31:01* were detected in Perm Krai and the Republics of Kalmykia, Buryatia, Tyva, and Sakha (Yakutia) (8.48%, $p = 0.042$; 8.79%, $p = 0.044$; 10.3%, $p = 3.4 \times 10^{-10}$; 20.44%, $p = 3.4 \times 10^{-10}$; 28.74%, $p = 5.4 \times 10^{-122}$, respectively, compared to a population frequency of 5.06%). The Republics of Dagestan, Ingushetia, and Kabardino-Balkaria showed a higher prevalence of impaired metabolism phenotype for phenytoin/phosphenytoin (46.4%, $p = 5.6 \times 10^{-36}$; 44.69%, $p = 1.7 \times 10^{-13}$; 43.83%, $p = 1.9 \times 10^{-16}$), primarily due to a high frequency of the *CYP2C9*3* allele. The Republics of Tatarstan, Mari El, and Chuvashia were also characterized by a high incidence of alleles associated with impaired metabolism of these drugs (37.06%, $p = 0.028$; 37.99%, $p = 0.031$; 41.2%, $p = 5.3 \times 10^{-10}$), attributable to the presence of the generally rare *CYP2C9*29* allele in their genetic structure.

Conclusions. The results obtained enable the formulation of region-specific recommendations for personalizing treatment with aromatic anticonvulsants. For residents of Sakha (Yakutia), Tyva, Buryatia, Kalmykia, and Perm Krai, testing for *HLA-A*31:01* carriage is justified. For residents of Tyva and Buryatia, additional testing for *HLA-B*15:11* carriage is warranted prior to the prescription of carbamazepine and oxcarbazepine. Before initiating phenytoin therapy, *CYP2C9* genotyping is particularly important for the populations of Dagestan, Ingushetia, Kabardino-Balkaria, Tatarstan, Mari El, and Chuvashia. However, this genotyping can be recommended for the entire population of Russia due to the high prevalence of alleles associated with reduced and absent enzyme activity.

Keywords: anticonvulsants; carbamazepine; lamotrigine; phenytoin; pharmacogenetics; HLA typing; *CYP2C9*; personalized medicine; Stevens–Johnson syndrome

For citation: Spektor E.D., Yudin V.S., Mamchur A.A., Rummyantseva A.M., Ivanov M.V., Mitrofanov S.I., Snigir E.A., Keskinov A.A., Yudin S.M., Kashtanova D.A. Pharmacogenetic factors determining the metabolism and safety of aromatic anticonvulsants in the residents of Russia. *Extreme Medicine*. 2026;28(1):51–60. <https://doi.org/10.47183/mes.2025-364>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study did not require an approval of a bioethics committee, as not involving direct interaction with participants and using secondary analysis of anonymized data. For the creation of the Population Frequency Database (GDB) by the Centre for Strategic Planning and Management of Biomedical Health Risks of the FMBA, voluntary informed consent for participation was obtained from all donors. The primary study was conducted in accordance with the principles of the Declaration of Helsinki.

Potential conflict of interest: Sergey M. Yudin is a member of the Editorial Board of *Extreme Medicine*. Anton A. Keskinov is the scientific editor of *Extreme Medicine*. The other authors declare no conflict of interest.

✉ Ekaterina D. Spektor espektor@cspfmba.ru

Received: 5 Aug. 2024 **Revised:** 25 Sep. 2025 **Accepted:** 22 Oct. 2025 **Online first:** 20 Nov. 2025

УДК 577.2

ФАРМАКОГЕНЕТИЧЕСКИЕ ФАКТОРЫ, ОПРЕДЕЛЯЮЩИЕ МЕТАБОЛИЗМ И БЕЗОПАСНОСТЬ АНТИКОНВУЛЬСАНТОВ С АРОМАТИЧЕСКОЙ СТРУКТУРОЙ У ЖИТЕЛЕЙ РОССИИ

Е.Д. Спектор[✉], В.С. Юдин, А.А. Мамчур, А.М. Румянцева, М.В. Иванов, С.И. Митрофанов, Е.А. Снигирь, А.А. Кескинов, С.М. Юдин, Д.А. Каштанова

Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

© E.D. Spektor, V.S. Yudin, A.A. Mamchur, A.M. Rummyantseva, M.V. Ivanov, S.I. Mitrofanov, E.A. Snigir, A.A. Keskinov, S.M. Yudin, D.A. Kashtanova, 2025

Введение. Применение антиконвульсантов с ароматической структурой (карбамазепин, окскарбазепин, ламотриджин, фенитоин) сопряжено с риском развития тяжелых реакций гиперчувствительности, которые отчасти зависят от генотипов *HLA-B* и *HLA-A* (для карбамазепина/окскарбазепина). Метаболизм фенитоина и фосфенитоина осуществляется с участием *CYP2C9*, и при генетически обусловленной замедленной трансформации препарата увеличивается вероятность развития нежелательных явлений. Учет генотипа *CYP2C9* позволяет персонализировать дозирование препарата и улучшить переносимость лечения.

Цель. Оценка распространенности основных фармакогенетических вариантов, ассоциированных с ответом на антиконвульсанты с ароматической структурой, с географической стратификацией и выделением популяций риска, в которых потенциально оправдана фармакогенетическая оценка при назначении препаратов данного класса.

Материалы и методы. Исследование выполнено на образцах из выборки базы данных популяционных частот (GDB) ЦСП ФМБА России ($n = 120\,979$, охват — 82 субъекта РФ). Проведено полногеномное секвенирование образцов ДНК с дальнейшим анализом распространенности носительства *HLA-B*15:02*, *HLA-B*15:11*, *HLA-A*31:01* и различных аллельных вариантов *CYP2C9* с расчетом индекса ферментной активности. Данные показатели сопоставлены в различных регионах РФ с выделением биогеографических групп риска.

Результаты. Вариант *HLA-B*15:02* имеет распространенность менее 1% во всех регионах РФ. В республиках Бурятия и Тыва наблюдается относительно высокая частота носительства *HLA-B*15:11* (1,3%, $p = 7,7 \times 10^{-5}$, и 3,46%, $p = 2,4 \times 10^{-3}$, соответственно, при популяционной 0,11%), в Пермском крае и республиках Калмыкия, Бурятия, Тыва, Саха — *HLA-A*31:01* (8,48%, $p = 0,042$; 8,79%, $p = 0,044$, 10,3%; $p = 3,4 \times 10^{-10}$; 20,44%, $p = 3,4 \times 10^{-10}$; 28,74%, $p = 5,4 \times 10^{-122}$, при популяционной 5,06%). В республиках Дагестан, Ингушетия, Кабардино-Балкария выше распространенность замедленного метаболизма фенитоина/фосфенитоина (46,4%, $p = 5,6 \times 10^{-36}$; 44,69%, $p = 1,7 \times 10^{-13}$; 43,83%, $p = 1,9 \times 10^{-16}$) за счет высокой распространенности *CYP2C9*3*. Республики Татарстан, Марий Эл, Чувашия также характеризуются высокой встречаемостью носителей аллелей замедленного метаболизма этих препаратов (37,06%, $p = 0,028$; 37,99%, $p = 0,031$; 41,2%, $p = 5,3 \times 10^{-10}$), но за счет наличия в генетической структуре повсеместно редкой аллели *CYP2C9*29*.

Выводы. Полученные результаты позволяют сформулировать регион-специфичные рекомендации по персонализации лечения антиконвульсантами с ароматической структурой. Для жителей Якутии, Тывы, Бурятии, Пермского края оправдано тестирование носительства *HLA-A*31:01*, для жителей Тывы и Бурятии — дополнительно носительства *HLA-B*15:11* перед назначением карбамазепина и окскарбазепина. Генотипирование *CYP2C9* перед началом терапии фенитоином особенно важно для населения республик Дагестан, Ингушетия, Кабардино-Балкария, Татарстан, Марий Эл, Чувашия, но в целом может быть рекомендовано всем жителям России ввиду высокой распространенности аллелей, ассоциированных со сниженной и отсутствующей ферментной активностью.

Ключевые слова: антиконвульсанты; карбамазепин; ламотриджин; фенитоин; фармакогенетика; HLA-типирование; *CYP2C9*; персонализированная медицина; синдром Стивенса – Джонсона

Для цитирования: Спектор Е.Д., Юдин В.С., Мамчур А.А., Румянцев А.М., Иванов М.В., Митрофанов С.И., Снигирь Е.А., Кескинов А.А., Юдин С.М., Каштанова Д.А. Фармакогенетические факторы, определяющие метаболизм и безопасность антиконвульсантов с ароматической структурой у жителей России. *Медицина экстремальных ситуаций*. 2026;28(1):51–60. <https://doi.org/10.47183/mes.2025-364>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование не требовало заключения биоэтического комитета, поскольку не предполагало непосредственного взаимодействия с участниками и проводилось на основе вторичного анализа деперсонифицированных данных. Для создания базы данных популяционных частот (GDB) ФГБУ «ЦСП» ФМБА России от всех участников получено добровольное информированное согласие на участие в исследовании, проведенном в соответствии с принципами Хельсинкской декларации.

Потенциальный конфликт интересов: С.М. Юдин — член редакционной коллегии журнала «Медицина экстремальных ситуаций», А.А. Кескинов — научный редактор журнала «Медицина экстремальных ситуаций», остальные авторы заявляют об отсутствии конфликта интересов.

✉ Спектор Екатерина Дмитриевна espektor@cspfmba.ru

Статья поступила: 05.08.2024 **После доработки:** 25.09.2025 **Принята к публикации:** 22.10.2025 **Online first:** 20.11.2025

INTRODUCTION

Antiepileptic drugs (AEDs), or anticonvulsants, are a class of pharmacological agents widely used in neurology and psychiatry. Their application extends beyond seizure control and includes the treatment of migraine,¹ cluster headache,² neuropathic pain,³ management of psychotic and behavioral disorders in elderly patients⁴ with cognitive impairment, as well as serving as mood stabilizers and anxiolytics [1]. Among these drugs, carbamazepine, oxcarbazepine, lamotrigine, and phenytoin have been extensively studied in terms of pharmacogenetic aspects related to their safety profiles. These drugs share a common structural feature — a benzene

ring — leading to their classification as “aromatic anti-convulsants” in the English-language literature [2]. The use of this drug class is associated with a low, yet clinically significant, risk of hypersensitivity reactions, such as severe cutaneous adverse reactions (SCARs), including epidermolytic drug reactions (e.g., Stevens–Johnson Syndrome (SJS), toxic epidermal necrolysis (TEN)), and systemic drug reactions with multiorgan involvement (e.g., Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)).

SJS and TEN are acute, severe allergic reactions characterized by extensive lesions of the skin and mucous membranes. They are currently viewed as different stages of the same pathological process, rather

¹ Migraine. Clinical guidelines. All-Russian Society of Neurologists. Russian Society for the Study of Headache (ROIGB); 2020 (In Russ.).

² Primary headaches: diagnosis and treatment. Methodological recommendations. Moscow City Health Department; 2017 (In Russ.).

³ Neuropathic pain: clinical guidelines for diagnosis and treatment of the Russian Society for the Study of Pain. Russian Society for the Study of Pain; 2018 (In Russ.).

⁴ Cognitive disorders in elderly and senile individuals. Russian Society of Psychiatrists; 2020 (In Russ.).

than as separate diseases [3, 4]. The incidence of SJS is 2.5–9.2 cases per million per year with a mortality rate of 4.1–16.9%. For TEN, the incidence amounts to 0.94–1.9 cases per million per year with a mortality rate of 14.8–29.9% [4]. The DRESS syndrome is characterized by widespread (> 50% of body surface area) exanthematous rash, fever, lymphadenopathy, eosinophilia, and internal organ involvement [5]. The exact incidence of DRESS is unknown, being estimated at one case per 10,000 drug exposures in the treated population. According to various estimates, the mortality rate ranges from 3.8% to 10–20% [6, 7]. Lamotrigine is among the top five drugs most strongly associated with fatal outcomes in the DRESS syndrome [8]. Furthermore, it ranks second among the medications most frequently implicated in the development of this syndrome [1].

Genetic predisposition plays a significant role in the likelihood of developing the aforementioned allergic reactions. Such predisposition is associated with the carriage of specific alleles of human leukocyte antigens A and B (*HLA-A*, *HLA-B*). These genes are part of a larger cluster known as the major histocompatibility complex. Accumulated evidence indicates the association between the development of SCAR/DRESS upon administration of phenytoin, lamotrigine, carbamazepine, and oxcarbazepine with the carriage of *HLA-B*15:02* (Level 1A) [9, 10]. Furthermore, the association between carbamazepine-induced SCAR/DRESS and the carriage of *HLA-A*31:01* (Level 1A), *HLA-B*15:11* (Level 1A), and *HLA-B*40:01* (Level 2A) was also confirmed [10, 11].

Currently, two primary sources provide pharmacogenetic recommendations to guide the prescription of aromatic anticonvulsants. These are the guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) [9, 11] and those from the Dutch Pharmacogenetics Working Group (DPWG) [10]. According to CPIC, carbamazepine, oxcarbazepine, or phenytoin should be avoided in carbamazepine-naïve patients who are carriers of the *HLA-B*15:02* allele. However, in cases where a patient has been using the target drug for three months or longer without developing cutaneous adverse reactions, the therapy may be continued regardless of *HLA-B* genotype. Indeed, SCARs typically manifest within the first 4–28 days of treatment [9, 11]. A similar decision-making algorithm is recommended for the prescription of carbamazepine in carriers of the *HLA-A*31:01* allele: avoid prescribing to carriers, except in situations where carbamazepine has been used continuously for three months or more without adverse effects [11].

The DPWG guidelines encompass a broader range of clinical scenarios. According to these recommendations, the use of lamotrigine is contraindicated in carriers of the *HLA-B*15:02* allele. Furthermore, the administration of carbamazepine is not recommended for carriers of the *HLA-B*15:02* and *HLA-A*31:01* alleles

(also indicated by CPIC), as well as for carriers of the *HLA-B*15:11* allele [10].

Phenytoin and its less commonly used prodrug — fosphenytoin — possess a narrow therapeutic index. Their tolerability profile, in addition to the hypersensitivity reactions discussed above, includes a wide spectrum of adverse effects, such as central nervous system toxicity, nausea, vomiting, gingival hyperplasia, osteomalacia, hematological suppression, and cognitive impairment [9]. The risk of developing these effects is largely determined by the rate of drug biotransformation, which is primarily mediated by the cytochrome P450 isoenzyme *CYP2C9*.

Numerous allelic variants of the *CYP2C9* gene exist, each conferring a predictable activity level of the encoded enzyme. The Activity Score (AS) system is used to describe the functional impact of a single allele (haplotype), classifying haplotypes as having normal (AS = 1), decreased (AS = 0.5), or zero (AS = 0) activity. Allele annotations are provided in the PharmGKB database [12]. The most common variants are *CYP2C9*1* (AS = 1), *CYP2C9*2* (AS = 0.5), and *CYP2C9*3* (AS = 0).

The clinically significant metabolic phenotype is determined based on the patient's diploid genotype, i.e., the sum of the AS values from both inherited alleles. According to international guidelines, this total score allows for classification into the following phenotypic groups: normal metabolizer (AS = 2), intermediate metabolizer ($1 \leq AS < 2$), and poor metabolizer (AS < 1). CPIC guidelines specify maintenance dose adjustments based on the metabolic phenotype: a 25% reduction of the standard maintenance dose is recommended for intermediate metabolizers with AS = 1, and a 50% reduction for poor metabolizers, while the loading dose regimen remains unchanged [9].

Therefore, when prescribing aromatic antiepileptic drugs, the pharmacogenetic profiles of patients should be taken into account. This enables risk management for serious hypersensitivity-mediated adverse reactions, prediction of individual drug metabolism rates, and development of personalized dosing regimens to improve treatment tolerability and its outcomes.

In this study, we aim to assess the population prevalence of major pharmacogenetic variants associated with response to aromatic antiepileptic drugs and to carry out their geographic stratification to identify high-risk populations where preemptive genotyping is clinically justified prior to initiating this drug class.

MATERIALS AND METHODS

Study object

The study was conducted using a population-based sample from the Population Frequency Database (GDB) of the Centre for Strategic Planning and Management of Biomedical Health Risks of the FMBA.⁵ The sample size

⁵ Population Genetic Variants Frequency Database of the Russian Federation Population. FMBA of Russia. App version 1.1.3 (17.03.2025). Database version 59.1 (03.10.2024) (In Russ.).

comprised 120,979 individuals (58,339 (48.2%) males, mean age 50.1 ± 14.1 years ($M \pm SD$)) from 82 federal subjects of the Russian Federation. For the analysis of regional allele frequency distribution, participants from the city of Moscow and Moscow Oblast, the city of Saint Petersburg and Leningrad Oblast, and the city of Sevastopol and the Republic of Crimea were pooled. The recruitment criteria for the GDB participants can be found in a previously published work [13]. For comparative analysis, regions with a sample size of 250 individuals or more were selected.

DNA extraction and sequencing

Genomic DNA was extracted from whole blood samples using MagAttract HMW DNA Kits (Qiagen, Germany) for high-molecular-weight DNA isolation with magnetic particles on automated liquid handling workstations. Whole-genome sequencing libraries were prepared using an Illumina DNA Prep reagent kit according to the manufacturer's protocol (Document No. 1000000025416 v10, Illumina, USA) with IDT-ILMN Nextera DNA UD Indexes Set A and Set B, followed by quality control. Whole-genome sequencing was performed on the Illumina NovaSeq 6000 platform using an S4 Reagent Kit (300 cycles) (Illumina, USA) for paired-end reads of 2×150 bp.

During the demultiplexing stage, sequencing data in BCL format were converted to FASTQ format using Illumina bcl2fastq2 v2.20.0.422.⁶ Quality control of the sequencing run was performed with Illumina Sequencing Analysis Viewer v2.4.7,⁷ while read quality (FASTQ.GZ format) was assessed using the FastQC v0.11.9 bioinformatic tool.⁸

Reads were aligned to the GRCh38.d1.vd1⁹ reference genome using the Illumina DRAGEN Bio-IT Platform v07.021.510.3.5.7.¹⁰ Quality control of the alignments (BAM format) was performed using DRAGEN, FastQC v0.11.9,¹¹ samtools v1.13 [14], and mosdepth v0.3.1 [15]. All samples passed quality control based on key metrics, including the percentage of duplicated reads, the number of unaligned reads, etc. The final mean genome coverage per sample was at least 30x.

Detection of small genetic variants (up to 50 bp) was performed using Illumina Strelka2 v2.9.10 [16]. The sample set was checked for duplicates using Picard CrosscheckFingerprints [17] with the built-in map file. The bioinformatic processing pipeline used was validated (F-score = 99.83%) against the HG001 reference genome provided by the Genome In A Bottle (GIAB) consortium (v.3.3.2) [18].

Pharmacogenetic variant screening and data statistical analysis

Selection of pharmacogenetic variants for analysis was based on their inclusion in existing clinical pharmacogenetic guidelines (CPIC or DPWG). The following allelic variants met this criterion: human leukocyte antigen alleles *HLA-B*15:02*, *HLA-B*15:11*, *HLA-A*31:01*, and variants of the cytochrome P450 isoenzyme gene *CYP2C9*. *CYP2C9* haplotyping was performed using the publicly available Panno algorithm.¹² Unphased HLA typing for six major loci was conducted using the xHLA [04.10.2017] (utilizing two IPD-IMGT/HLA database versions: 3.22.0 [10.2015] and 3.45.1 [08.2021]) and HLAscan v2.1.4 [04.12.2019] (IPD-IMGT/HLA database version 3.21.0 [07.2015]) bioinformatic tools.

For each allele, the carrier frequency and 95% confidence interval (CI), calculated using the Wilson method, were determined. Based on the *CYP2C9* allele annotations provided in PharmGKB [13], a *CYP2C9* Activity Score (AS) was calculated for each sample. The corresponding metabolic phenotype for enzyme substrates was subsequently assigned based on the AS according to established algorithms. The proportion of individuals in the population requiring personalized phenytoin/fosphenytoin dosing per CPIC guidelines [9] was calculated, along with the combined proportion of intermediate and poor metabolizers, subsequently designated as the "impaired metabolism" group.

Statistical analysis was performed using the Python programming language, version 3.9. A z-test for proportions was used to compare the carrier frequencies of HLA alleles and the proportions of *CYP2C9* substrate impaired metabolizers between regions and the overall population values. The Benjamini-Hochberg procedure, controlling the False Discovery Rate (FDR) at 5%, was applied to correct for multiple testing. Associations were considered statistically significant either at a *p*-value < 0.05 or by the interval method when the confidence interval did not cross a predefined critical threshold.

RESULTS

Severe risk of hypersensitivity reaction in the population treated with aromatic antiepileptic drugs

The absolute counts and percentage frequencies of the risk alleles *HLA-B*15:02*, *HLA-B*15:11*, and *HLA-A*31:01* were 22 (0.01%), 134 (0.06%), and 6122 (2.53%), respectively. The estimated carrier frequencies for these alleles were 0.02% (*HLA-B*15:02*), 0.11% (*HLA-B*15:11*),

⁶ bcl2fastq and bcl2fastq2 Conversion Software Downloads. URL: https://emea.support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software/downloads.html

⁷ Sequencing Analysis Viewer Support. URL: https://support.illumina.com/sequencing/sequencing_software/sequencing_analysis_viewer_sav.html

⁸ Babraham Bioinformatics — FastQC A Quality Control tool for High Throughput Sequence Data. URL: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

⁹ GDC Reference Files | NCI Genomic Data Commons. URL: <https://gdc.cancer.gov/about-data/gdc-data-processing/gdc-reference-files>

¹⁰ DRAGEN secondary analysis | Software for NGS data analysis. URL: <https://www.illumina.com/products/by-type/informatics-products/dragen-secondary-analysis.html>

¹¹ Babraham Bioinformatics — FastQC A Quality Control tool for High Throughput Sequence Data. URL: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

¹² PAnno: A Pharmacogenomics Annotation Tool for Clinical Genomic Testing. URL: <https://github.com/PreMedKB/PAnno.git>

and 5.06% (*HLA-A*31:01*). The presence of at least one risk allele was observed in 5.18% of the population. This was primarily driven by the *HLA-A*31:01* allele, since the other alleles are extremely rare in the general population. Analysis of the regional distribution of risk allele carriers revealed regions with both statistically significantly lower and higher carrier frequencies compared to the overall population. Table 1 presents data for regions where the frequency of risk alleles for SCAR/DRESS was significantly higher than the population average.

The *HLA-B*15:02* allele exhibits an extremely low prevalence in the general population and was not detected in 53 out of 68 regions. The highest frequency nationwide was observed in Kalmykia (0.52%, $p\text{-adj} = 0.21$).

For the *HLA-B*15:11* and *HLA-A*31:01* alleles, distinct high-risk regions can be identified, which partially overlap (Table 1). The *HLA-B*15:11* variant was not detected in 33 regions. A regional risk assessment for carrying at least one of these risk alleles is illustrated in Figure 1. Based on interval estimation, the republics of Sakha (Yakutia), Tyva, Buryatia, Kalmykia, and Perm Krai demonstrate a statistically significant elevated risk of carriage for the *HLA-B*15:02*, *HLA-B*15:11*, or *HLA-A*31:01* alleles.

Phenytoin metabolism in the population

CYP2C9 genotype data were available for 119,422 participants (48.4% males). Figure 2 presents the frequency distributions of the most common alleles, enzyme activity scores, and metabolic phenotypes in the population. The *1 (reference), *2, and *3 allelic variants account for 99% of all detected alleles (Fig. 2A).

Approximately one-third (33.6%) of the population exhibits impaired (intermediate or poor) metabolism of

CYP2C9 substrates, although the poor metabolizer phenotype is relatively rare (2% of cases). Table 2 presents data for regions with statistically significant higher or lower prevalence of impaired metabolizers of *CYP2C9* substrates.

Several republics in the North Caucasus (Kabardino-Balkaria, Ingushetia, and Dagestan), along with the republics of Tatarstan, Mari El, and Chuvashia, can be characterized as high-risk regions for poor tolerability during treatment with phenytoin/fosphenytoin, as well as other *CYP2C9* substrate medications not examined in the present study. Conversely, in Sakha (Yakutia), Tyva, Kalmykia, Buryatia, and the Kemerovo region, reduced *CYP2C9* enzyme activity is relatively uncommon.

To elucidate the underlying causes of the differences between high-risk regions and the general population, we analyzed the structure of *CYP2C9* allelic variants. The observed disparities could be attributed either to an increased frequency of the *2 and *3 alleles or to the presence of region-specific rare alleles. Variants with a prevalence exceeding 1% in any given region are presented in Table 3 alongside the corresponding population-wide frequencies.

Thus, the increased frequency of genotypes associated with impaired metabolism of *CYP2C9* substrates in Kabardino-Balkaria, Ingushetia, and Dagestan is driven by a higher prevalence of the *CYP2C9**3 variant, which corresponds to severely reduced enzyme activity. A different pattern is observed in Tatarstan, Mari El, and Chuvashia. Here, while the frequencies of the *2 and *3 alleles are comparable to the general population, the primary contributing factor is the relatively high prevalence of the *29 allele, which also confers reduced enzyme activity.

Table 1. High-prevalence regions for risk alleles causing drug-induced epidermolysis and DRESS syndrome

Allelic variant	Carrier status in the population, %	Regions with higher prevalence	Carrier status in the region, %	$p\text{-adj}$
<i>HLA-B*15:02</i>	0.02	no region	N/A	N/A
<i>HLA-B*15:11</i>	0.11	Buryatia	1.3%	7.7×10^{-5}
		Tyva	3.46%	2.4×10^{-3}
		Sakha (Yakutia)	0.68%	3.2×10^{-3}
<i>HLA-A*31:01</i>	5.06	Perm Krai	8.48%	0.042
		Kalmykia	8.79%	0.044
		Buryatia	10.3%	3.4×10^{-10}
		Tyva	20.44%	3.4×10^{-10}
		Sakha (Yakutia)	28.74%	5.4×10^{-122}

Table compiled by the authors based on original data

Note: $p\text{-adj}$ — FDR-corrected p -value (Benjamini–Hochberg false discovery rate); N/A — not applicable.

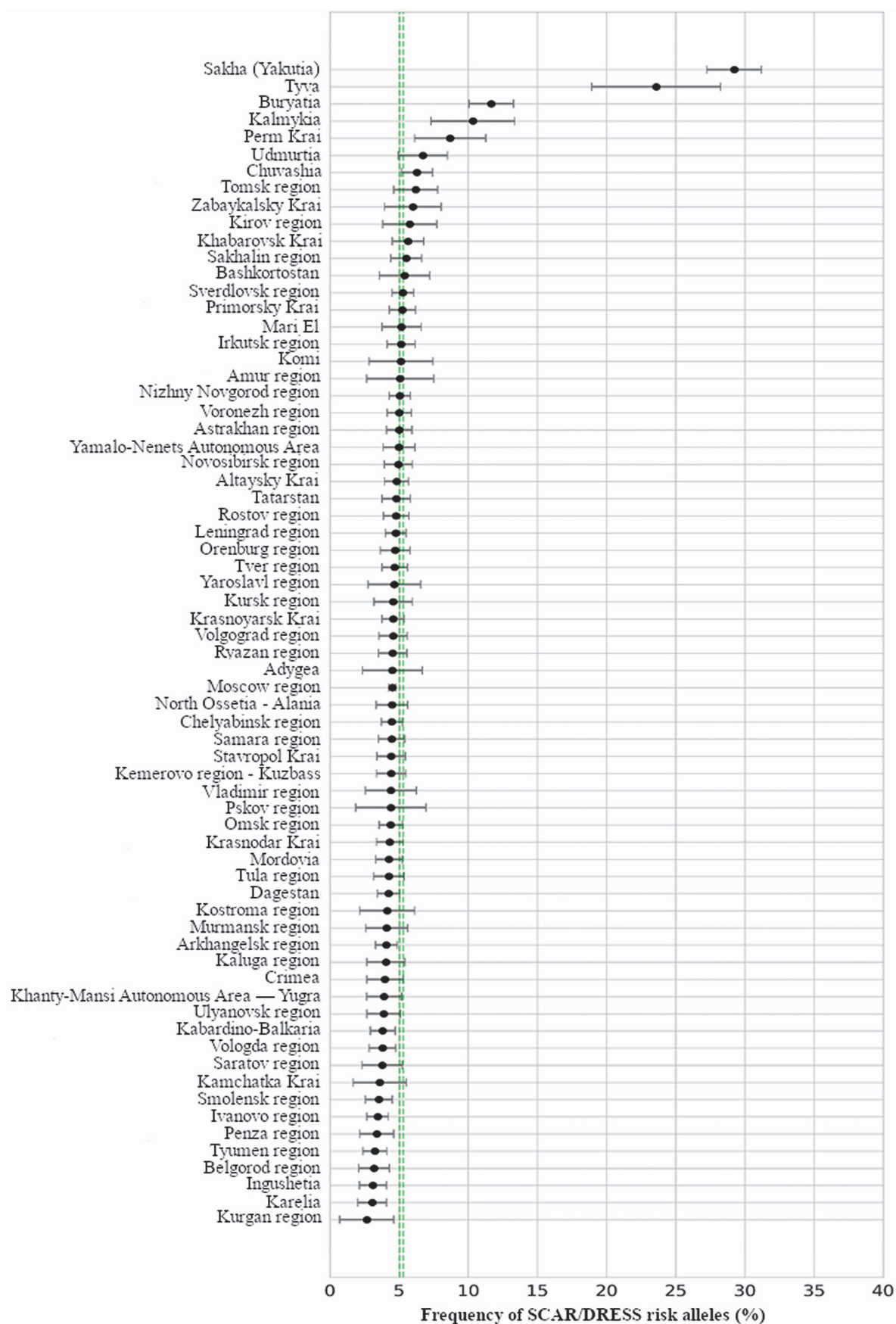


Figure prepared by the authors based on original data

Fig. 1. Allele frequency of risk variants (*HLA-B*15:02*, *HLA-B*15:11*, or *HLA-A*31:01* combined) in regions of the Russian Federation: the double dashed line represents the 95% confidence interval (CI) for the population-wide prevalence

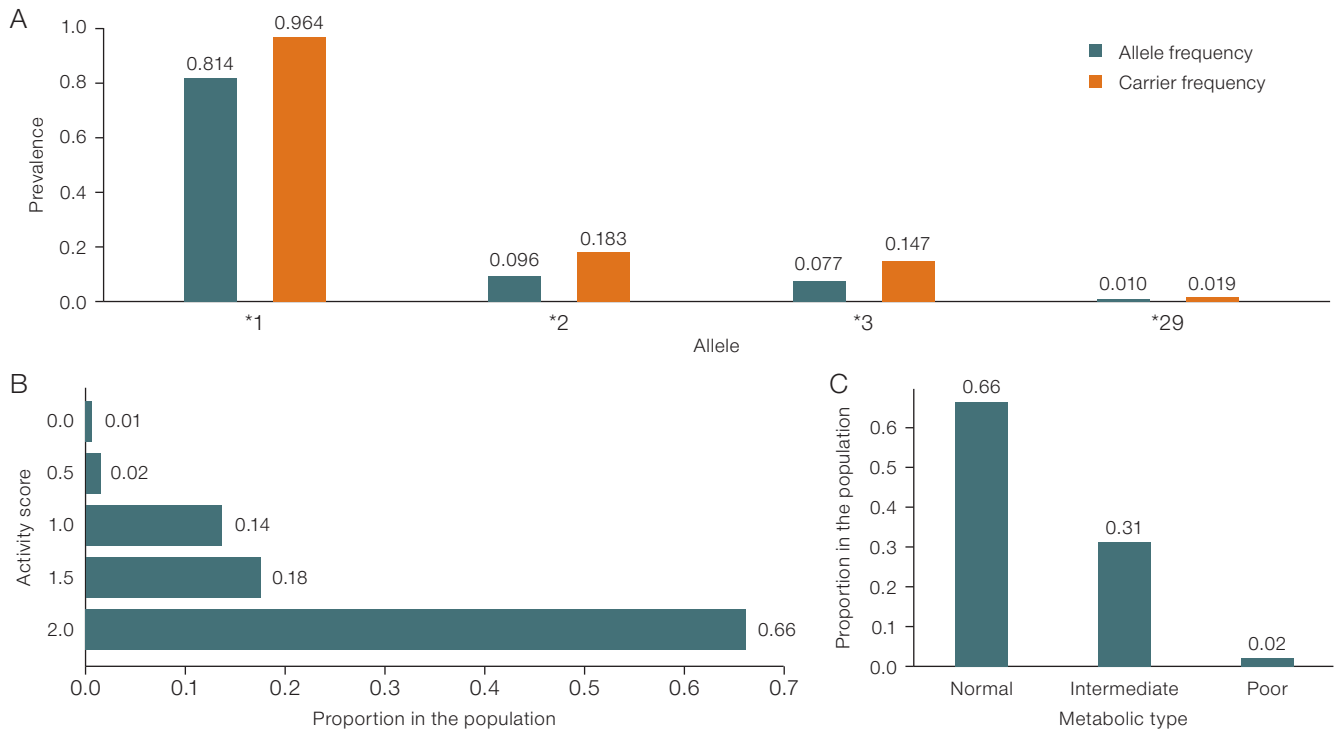


Figure prepared by the authors based on original data

Fig. 2. Population-Wide Characterization of *CYP2C9* Genetics: A — prevalence of the most frequent alleles; B — distribution of the genotype-predicted activity score; C — distribution of metabolic phenotypes

DISCUSSION

Our results have enabled the identification of both population-wide trends and risks associated with the use of AEDs, as well as the stratification of the population to delineate biogeographic groups requiring particular caution when prescribing aromatic anticonvulsants.

Overall, the population of the Russian Federation can be characterized as having a low risk of developing severe toxic-allergic reactions, such as Stevens–Johnson syndrome, toxic epidermal necrolysis, and DRESS syndrome, when using aromatic AEDs. However, a moderate risk of an intermediate phenytoin metabolism phenotype has been established, necessitating a corresponding 25% reduction in the drug maintenance dose [9]. Failure to account for this factor may not only worsen treatment tolerability and increase temporal and financial costs associated with switching AEDs, but also lead to the development of drug resistance [19].

The populations in such regions as Sakha (Yakutia), Tyva, Buryatia, Kalmykia, and to a lesser extent Perm Krai, can be identified as high-risk populations for carrying allelic variants associated with severe hypersensitivity reactions to carbamazepine and oxcarbazepine. This is due to the relatively high prevalence of *HLA-B*15:11* and *HLA-A*31:01* variants. However, this conclusion cannot be extended to lamotrigine and phenytoin, since the only reliably established association for hypersensitivity reactions to these drugs currently exists with the *HLA-B*15:02* allele. This allele has a frequency of less than 1% across all biogeographic groups in Russia.

According to the literature, *HLA-B*15:02* and *HLA-A*31:01* alleles are globally rare, yet their carrier frequency exhibits its ancestry dependence. An elevated frequency of the *HLA-B*15:02* variant has been described in indigenous populations of East Asia (6.9%), Australia and Oceania (5.4%), and South/Central Asia (4.6%). Similarly, the *HLA-A*31:01* variant is more prevalent in individuals of European ancestry (3%), Hispanic populations in South America (6%), and some East Asian populations (up to 8%) [11].

Notably, the high-risk regions for SCAR/DRESS identified in our study are heterogeneous in terms of the specific alleles driving this risk. For the prevention of severe hypersensitivity reactions to carbamazepine/oxcarbazepine, preemptive testing for *HLA-A*31:01* carriage appears sufficient in the republics of Sakha (Yakutia), Kalmykia, and Perm Krai. In contrast, in the republics of Tyva and Buryatia, testing for both *HLA-B*15:11* and *HLA-A*31:01* is warranted, with subsequent clinical decisions guided by current CPIC guidelines [11]. However, precise determination of the optimal diagnostic panel requires further health-economic analysis.

The structure of *CYP2C9* allelic variants in the Russian population highlights its distinct characteristics. On the one hand, the leading allelic variants, aside from the wild-type (*1), are *2 and *3, which aligns with the global pattern and are included in the recommended diagnostic genotyping panel for this enzyme [21]. On the other hand, the frequency of *CYP2C9*2* (0.096) is somewhat higher than in the European population (0.076), most closely resembling the corresponding value in Middle Eastern populations (0.083) [12, 21].

Table 2. Comparative analysis of the prevalence of different *CYP2C9* substrate metabolism phenotypes in regions with deviating frequencies of slow metabolizers (intermediate + poor) from the general population

Direction of differences between regional and population estimates	Region	Prevalence, %			p-adj
		Prevalence of impaired metabolism (intermediate + poor)	Prevalence of intermediate metabolism phenotype (AS 1–1.5)	Prevalence of poor metabolizer phenotype (AS 0–0.5)	
The proportion of impaired metabolizers is lower than the population value	Sakha (Yakutia)	17.6	17.01	0.59	2.7×10 ⁻⁷⁸
	Tyva	17.98	17.35	0.63	5.9×10 ⁻¹²
	Kalmykia	21.3	19.22	2.08	3.6×10 ⁻⁸
	Buryatia	21.98	21.2	0.78	1.7×10 ⁻²⁶
	Kemerovo region	30.11	28.57	1.54	0.031
The proportion of impaired metabolizers is higher than the population average	Tatarstan	37.06	34.56	2.5	0.028
	Mari-El	37.99	34.6	3.39	0.031
	Chuvashia	41.2	38.06	3.14	5.3×10 ⁻¹⁰
	Kabardino-Balkaria	43.83	39.32	4.51	1.9×10 ⁻¹⁶
	Ingushetia	44.69	38.53	6.16	1.7×10 ⁻¹³
	Dagestan	46.4	39.64	6.76	5.6×10 ⁻³⁶

Table compiled by the authors based on original data

Note: p-adj — FDR-adjusted p-value (Benjamini-Hochberg false discovery rate) for comparison of the total proportion of impaired metabolizers (intermediate + poor) with the population value.

Concurrently, nearly 2% of the Russian population are carriers of the *CYP2C9**29 allele, which is exceptionally rare across all biogeographic groups [21]. This allele encodes an enzyme with reduced activity (AS = 0.5) yet is not included in the extended diagnostic panel recommended by international guidelines [20].

The analysis of the prevalence of genetically determined impaired phenytoin metabolism has demonstrated that the overall Russian population is characterized by a high prevalence (33.6%) of this phenotype. This necessitates consideration not only when prescribing phenytoin/fosphenytoin but also for warfarin [22], a number of non-steroidal anti-inflammatory drugs [24], and other medications whose biotransformation involves the *CYP2C9* isoenzyme.

The issue of reduced *CYP2C9* enzyme activity is particularly relevant in the Republics of Tatarstan, Mari El, Chuvashia, Kabardino-Balkaria, Ingushetia, and Dagestan, where the frequency of genotypes conferring reduced *CYP2C9* activity exceeds the population average, reaching 37–46%. However, the underlying genetic source differs fundamentally between the group of North Caucasian republics and Tatarstan, Mari El, and Chuvashia.

In the former case, the distribution of common allelic variants is shifted toward a predominance of the

*CYP2C9**3 allele, which corresponds to severely reduced enzyme activity. Therefore, for residents of Kabardino-Balkaria, Ingushetia, and Dagestan, detection of *CYP2C9**2 and *CYP2C9**3 alleles can be recommended for personalizing therapy with phenytoin/fosphenytoin and other drugs metabolized by *CYP2C9*.

In Chuvashia, Tatarstan, and Mari El, the deviation from the population average can be described as qualitative, since the risk of impaired metabolism is driven by a different allele — *CYP2C9**29 — which is not widely prevalent in the general population. For this reason, inclusion of this variant in a region-specific diagnostic panel for these areas appears promising. However, similar to HLA typing, the cost-effectiveness of implementing such diagnostic technologies can only be clarified following a formal health-economic analysis.

CONCLUSIONS

Our study demonstrates significant variability in the prevalence of genetic markers associated with impaired metabolism and the risk of adverse reactions to aromatic anticonvulsants among the population of the Russian Federation. While carriage of alleles increasing the risk of SCAR/DRESS is rare in the general population, the high prevalence of some of them (*HLA-B**15:11

Table 3. Frequency of *CYP2C9* allelic variants in regions with elevated prevalence of genotypes associated with impaired metabolism of enzyme substrates

Region	<i>n</i>	Allelic variant	Regional frequency, %	Population frequency, %	Statistical significance level, <i>p</i>
Tatarstan	1600	*2	9.53	9.62	0.87
		*3	8.59	7.7	0.17
		*29	2.16	0.99	0.002
Mari-El	954	*2	7.94	9.62	0.074
		*3	7.94	7.7	0.74
		*29	5.6	0.99	4.2×10⁻⁹
Chuvashia	1779	*2	7.81	9.62	0.005
		*3	8.89	7.7	0.07
		*29	6.32	0.99	1.8×10⁻²⁰
Kabardino-Balkaria	1709	*2	11.43	9.62	0.02
		*3	12.7	7.7	3.9×10⁻¹⁰
Ingushetia	1186	*2	7.12	9.62	0.001
		*3	18.25	7.7	4.7×10⁻²¹
Dagestan	2470	*2	8.52	9.62	0.056
		*3	17.87	7.7	3.4×10⁻⁴⁰

Table compiled by the authors based on original data

Note: the presented alleles have a regional frequency of > 1%; *N* — number of observations in the region; statistically significant differences (*p* < 0.05) are highlighted in bold.

and *HLA-A*31:01*) among residents of Sakha (Yakutia), Tyva, Buryatia, Kalmykia, and Perm Krai warrants recommending genetic testing for these variants prior to prescribing carbamazepine and oxcarbazepine in these regions.

The use of lamotrigine appears equally safe across all biogeographic groups of the Russian population due to the low prevalence of the *HLA-B*15:02* allele.

Regarding phenytoin, approximately one-third of the population has a genetically determined impaired metabolism of the drug, requiring dose adjustment, particularly in the republics of the North Caucasus, as well as in Tatarstan, Mari El, and Chuvashia. Consequently, before initiating phenytoin therapy, *CYP2C9* genotyping is especially important for the population of these regions but can generally be recommended for all residents of Russia.

References

1.

Żelabowski K, Wojtyshiak K, Ratka Z, Biedka K, Chlopas-Konowalek A. Lamotrigine Therapy: Relation Between Treatment of Bipolar Affective Disorder and Incidence of Stevens–Johnson Syndrome—A Narrative Review of the Existing Literature. *Journal of Clinical Medicine*. 2025;14(12):4103.
<https://doi.org/10.3390/jcm14124103>

2.

Ahmed AF, Sukasem C, Sabbah MA, Musa NF, Noor DAM, Daud NAA. Genetic Determinants in HLA and Cytochrome P450 Genes in the Risk of Aromatic Antiepileptic-Induced Severe Cutaneous Adverse Reactions. *Journal of Personalized Medicine*. 2021;11(5):383.
<https://doi.org/10.3390/jpm11050383>

3.

Hasegawa A, Abe R. Recent advances in managing and understanding Stevens–Johnson syndrome and toxic epidermal necrolysis. *F1000Research*. 2020;9:F1000 Faculty Rev-612.
<https://doi.org/10.12688/f1000research.24748.1>

4.

Watanabe Y, Hama N. Recent advances in the diagnosis and treatment of Stevens–Johnson syndrome/toxic epidermal necrolysis. *Allergology International*. 2025;74(3):345–55.
<https://doi.org/10.1016/j.alit.2025.05.008>

5.

Mori F, Caffarelli C, Caimmi S, Bottau P, Liotti L, Franceschini F, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS) in children. *Acta Biomedica*. 2019;90(3-S):66–79.

6.

Calle AM, Aguirre N, Ardila JC, Villa RC. DRESS syndrome: A literature review and treatment algorithm. *World Allergy Organization Journal*. 2023;16(3):100673.
<https://doi.org/10.1016/j.waojou.2022.100673>

7.

López-Rocha E, Blancas L, Rodríguez-Mireles K, Gaspar-Lopez A, O’Farrill-Romanillos P, Amaya-Mejia A, et al. Prevalence of DRESS syndrome. *Revista Alergia Mexico*. 2014;61(1):14–23.

8.

Liang C, An P, Zhang Y, Liu X, Zhang B. Fatal outcome related to drug reaction with eosinophilia and systemic symptoms: a disproportionality analysis of FAERS database and a systematic review of cases. *Frontiers in Immunology*. 2024;15:1490334.
<https://doi.org/10.3389/fimmu.2024.1490334>

9.

Karnes JH, Rettie AE, Somogyi AA, Huddart R, Fohner AE, Formea CM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *CYP2C9* and *HLA-B*

- Genotypes and Phenytoin Dosing: 2020 Update. *Clinical Pharmacology and Therapeutics*. 2021;109(2):302–9. <https://doi.org/10.1002/cpt.2008>
10. Manson LEN, Nijenhuis M, Soree B, de Boer-Veger NJ, Buunk AM, Houwink EJJ, et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of CYP2C9, HLA-A and HLA-B with anti-epileptic drugs. *European Journal of Human Genetics*. 2024;32(8):903–11. <https://doi.org/10.1038/s41431-024-01572-4>
 11. Phillips EJ, Sukasem C, Whirl-Carrillo M, Muller DJ, Dunnenberger HM, Chantratita W, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. *Clinical Pharmacology and Therapeutics*. 2018;103(4):574–81. <https://doi.org/10.1002/cpt.1004>
 12. Barbarino JM, Whirl-Carrillo M, Altman RB, Klein TE. PharmGKB: A worldwide resource for pharmacogenomic information. *WIREs*. 2018;10(4):e1417. <https://doi.org/10.1002/wsbm.1417>
 13. Gusakova M, Dzhumaniazova I, Zelenova E, Kashtanova D, Ivanov M, Mamchur A, et al. Prevalence of the cancer-associated germline variants in Russian adults and long-living individuals: using the ACMG recommendations and computational interpreters for pathogenicity assessment. *Frontiers in Oncology*. 2024;14:1420176. <https://doi.org/10.3389/fonc.2024.1420176>
 14. Li H, Handsaker B, Wysoker A, Fennel T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078. <https://doi.org/10.1093/bioinformatics/btp352>
 15. Pedersen BS, Quinlan AR. Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics*. 2018;34(5):867–8. <https://doi.org/10.1093/bioinformatics/btx699>
 16. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Kallberg M, et al. Strelka2: fast and accurate calling of germline and somatic variants. *Nature Methods*. 2018;15(8):591–4. <https://doi.org/10.1038/s41592-018-0051-x>
 17. Najafov J, Najafov A. CrossCheck: an open-source web tool for high-throughput screen data analysis. *Scientific Reports*. 2017;7(1):5855. <https://doi.org/10.1038/s41598-017-05960-3>
 18. Krusche P, Trigg L, Boutros PC, Mason CE, De La Vega FM, Moore BL, et al. Best practices for benchmarking germline small-variant calls in human genomes. *Nature Biotechnology*. 2019;37(5):555–60. <https://doi.org/10.1038/s41587-019-0054-x>
 19. Finamore JM, Sperling MR, Zhan T, Nei M, Skidmore CT, Mintzer S. Seizure outcome after switching antiepileptic drugs: A matched, prospective study. *Epilepsia*. 2016;57(8):1294–300. <https://doi.org/10.1111/epi.13435>
 20. Pratt VM, Cavallari LH, Del Tredici AL, Hachad H, Ji Y, Moyer AM, et al. Recommendations for Clinical CYP2C9 Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists. *Journal of Molecular Diagnostics*. 2019;21(5):746–55. <https://doi.org/10.1016/j.jmoldx.2019.04.003>
 21. Huddart R, Fohner AE, Whirl-Carrillo M, Wojcik GL, Gignoux CR, Popejoy AB, et al. Standardized Biogeographic Grouping System for Annotating Populations in Pharmacogenetic Research. *Clinical Pharmacology and Therapeutics*. 2019;105(5):1256–62. <https://doi.org/10.1002/cpt.1322>
 22. Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. *Clinical Pharmacology and Therapeutics*. 2017;102(3):397–404. <https://doi.org/10.1002/cpt.668>
 23. Theken KN, Lee CR, Gong L, Caudle KE, Formea CM, Gaedigk A, et al. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and Nonsteroidal Anti-Inflammatory Drugs. *Clinical Pharmacology and Therapeutics*. 2020;108(2):191–200. <https://doi.org/10.1002/cpt.1830>

Authors' contributions. All authors confirm that they meet the ICMJE criteria for authorship. The largest contribution was distributed as follows: Ekaterina D. Spektor — conceptualization, formal analysis; Vladimir S. Yudin — methodology, manuscript drafting and editing; Aleksandra A. Mamchur — manuscript drafting; Antonina M. Rummyantseva — resources; Mikhail V. Ivanov — software; Sergey I. Mitrofanov — data verification; Ekaterina A. Snigir — conducting research; Anton A. Keskinov — project administration, management of planning and implementation of research activities; Sergey M. Yudin — research management; Daria A. Kashtanova — coordination of planning and implementation of research activities.

AUTHORS

Ekaterina D. Spektor, Cand. Sci. (Med.)
<https://orcid.org/0000-0003-0714-9476>
ESpektor@cspfmmba.ru

Vladimir S. Yudin, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-9199-6258>
VYudin@cspfmmba.ru

Aleksandra A. Mamchur
<https://orcid.org/0000-0002-6025-7663>
AMamchur@cspfmmba.ru

Antonina M. Rummyantseva
<https://orcid.org/0009-0006-4830-8057>
Rummyantseva@cspfmmba.ru

Mikhail V. Ivanov
<https://orcid.org/0009-0004-7070-5636>
MIvanov@cspfmmba.ru

Sergey I. Mitrofanov, Cand. Sci. (Biol.)
<https://orcid.org/0000-0003-0358-0568>
Mitrofanov@cspfmmba.ru

Ekaterina A. Snigir, Cand. Sci. (Biol.)
<https://orcid.org/0000-0003-1245-7764>
ESnigir@cspfmmba.ru

Anton A. Keskinov, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7378-983X>
Keskinov@cspfmmba.ru

Sergey M. Yudin, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-7942-8004>
info@cspfmmba.ru

Daria A. Kashtanova, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-8977-4384>
DKashtanova@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-324>



OPTIMIZATION OF INITIAL CULTURE STAGE OF VERO AND HEK293 CELL LINES

Irina I. Tuzova[✉], Tatyana I. Chirkina, Igor A. Churkin, Anastasia N. Lyakh, Kirill M. Mefed, Vladimir A. Maximov

Centre for Strategic Planning of the Federal Medical and Biological Agency, Moscow, Russia

Introduction. Laboratory production of viral material in small quantities is performed, as a rule, using adherent cell lines and culture flasks of varying surface area. The need to increase product yield leads to either an increase in the number of flasks or a switch to other accumulation systems, such as roller bottles. One factor influencing the efficiency of cell adhesion and homogeneous monolayer formation is the rotation frequency of the roller bottle. There is a lack of available research data on the impact of rotation frequency on these parameters and determination of its optimal value, particularly based on cellular morphology.

Objective. To optimize the initial stage of roller cultivation for Vero and HEK293 cell lines, taking into account the effect of roller bottle rotation frequency on cell adhesion during seeding and monolayer formation.

Materials and methods. Experiments were conducted using two monolayer cell lines, Vero and HEK293. Seeding concentrations were taken from the cell line passports, amounting to 4×10^4 cells/cm². Each cell line was seeded onto roller bottles and cultured according to the range of rotation frequencies (0.2, 0.3, 0.4, 0.5, and 0.6 rpm) using a Celrol Mid roller (Wiggins) in a RWD D180 CO₂ incubator. Following 1, 2, and 3 days of cultivation, the quality of cell adherence to the growth surface and monolayer formation was assessed by a TC5400 microscope (Meiji Techno).

Results. During cultivation of the Vero cell line, the rotation frequency up to 0.6 rpm did not significantly affect cell adhesion to the surface. The most homogenous cell distribution was observed at rotation frequencies of 0.4–0.5 rpm. The HEK293 cell culture is more sensitive to mechanical disturbances of the nutrient medium; as a result, at rotation frequencies above 0.2 rpm, abnormally rounded cell shapes and impaired adherence to the growth surface were observed. Furthermore, continued cultivation at this rotation frequency did not lead to the formation of a homogenous monolayer due to slow alternation between the respiration and nutrition phases. Consequently, after cell adherence to the surface, the rotation frequency of the roller bottle should be increased.

Conclusions. For the Vero cell line, the optimal rotation frequency was established to be 0.4–0.5 rpm. For the HEK293 cell line, the rotation frequency should be at least 0.2 rpm during the first day followed by its increase to 0.5 rpm after 24 h. The tested cultivation conditions enable an efficient growth of these cell lines for the production of viral biomass.

Keywords: cell cultures; cell cultivation; adherent cultures; Vero cell line; HEK293 cell line; roller bottles; roller cultivation; adhesion; monolayer formation; cell morphology; cell biomass production

For citation: Tuzova I.I., Chirkina T.I., Churkin I.A., Lyakh A.N., Mefed K.M., Maximov V.A. Optimization of initial culture stage of Vero and HEK293 cell lines. *Extreme Medicine*. 2026;28(1):61–68. <https://doi.org/10.47183/mes.2025-324>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study did not require approval from a local bioethics committee. HEK293 and Vero cell lines were provided by the Institute of Synthetic Biology and Genetic Engineering of Centre for Strategic Planning of FMBA.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Irina I. Tuzova Ituzova@cspfmbs.ru

Received: 4 June 2025 **Revised:** 3 Sep. 2025 **Accepted:** 1 Oct. 2025 **Online first:** 27 Oct. 2025

УДК 576.524

ОПТИМИЗАЦИЯ НАЧАЛЬНОГО ЭТАПА КУЛЬТИВИРОВАНИЯ КЛЕТОЧНЫХ ЛИНИЙ VERO И HEK293

И.И. Тузова[✉], Т.И. Чиркина, И.А. Чуркин, А.Н. Лях, К.М. Мефёд, В.А. Максимов

Центр стратегического планирования и управления медико-биологическими рисками здоровью Федерального медико-биологического агентства, Москва, Россия

Введение. Нарботка в лабораториях вирусного материала в малых количествах, как правило, проводится с использованием адгерентных линий и культуральных флаконов различной площади. Необходимость увеличения выхода продукта приводит или к увеличению количества флаконов, или смене системы накопления, например на роллерные бутылки. Одним из факторов, оказывающих влияние на эффективность адгезии клеток и формирование однородного монослоя, является частота вращения роллерной бутылки. При этом отмечено небольшое количество исследований, касающихся оценки влияния частоты вращения и определения ее оптимального показателя, особенно на основе морфологии клеток.

Цель. Оптимизировать начальный этап роллерного культивирования клеточных линий Vero и HEK293 с учетом влияния частоты вращения роллерной бутылки на прикрепление клеток при посеве и формирование монослоя.

Материалы и методы. Для проведения экспериментальной работы были использованы 2 монослойные клеточные линии: Vero и HEK293. Посевные концентрации были взяты из паспортов клеточных линий и составляли 4×10^4 кл/см². Клеточную линию засевали на роллерные бутылки и культивировали согласно диапазону частот вращения 0,2, 0,3, 0,4, 0,5 и 0,6 об/мин с использованием роллерной установки Celrol Mid, Wiggins в CO₂-инкубаторе D180, RWD. Через 1, 2 и 3 сут культивирования оценивали качество прикрепления клеток к ростовой поверхности и формирование монослоя путем просмотра под микроскопом TC5400, Meiji Techno.

© I.I. Tuzova, T.I. Chirkina, I.A. Churkin, A.N. Lyakh, K.M. Mefed, V.A. Maximov, 2025

Результаты. При культивировании клеточной линии Vero частота вращения до 0,6 об/мин не оказывала значительного влияния на адгезию клеток к поверхности. Наиболее равномерное расположение клеток наблюдали при частоте вращения 0,4–0,5 об/мин. Культура клеток HEK293 более чувствительна к механическим воздействиям питательной среды, и при частоте вращения свыше 0,2 об/мин наблюдали атипично округлые формы клеток и нарушение полноценного их прикрепления к ростовой поверхности. При этом дальнейшее культивирование на данной частоте вращения не приводило к формированию однородного монослоя из-за медленного чередования «фазы дыхания» и «фазы питания». Следовательно, после прикрепления клеток к поверхности необходимо увеличение частоты вращения роллерной бутыли.

Выводы. Для клеточной линии Vero оптимальной частотой вращения является 0,4–0,5 об/мин, для клеточной линии HEK293 в первые сутки необходимо устанавливать 0,2 об/мин, а через сутки увеличить до 0,5 об/мин. Апробированные условия культивирования позволяют выращивать данные клеточные линии для наработки вирусной биомассы.

Ключевые слова: культуры клеток; культивирование клеток; адгерентные культуры; клеточная линия Vero; клеточная линия HEK293; роллерные бутыли; роллерное культивирование; адгезия; формирование монослоя; морфология клеток; наработка клеточной биомассы

Для цитирования: Тузова И.И., Чиркина Т.И., Чуркин И.А., Лях А.Н., Мефёд К.М., Максимов В.А. Оптимизация начального этапа культивирования клеточных линий Vero и HEK293. *Медицина экстремальных ситуаций*. 2026;28(1):61–68. <https://doi.org/10.47183/mes.2025-324>

Финансирование: работа выполнена без спонсорской поддержки.

Соответствие принципам этики: исследование не требовало заключения локального биоэтического комитета. В работе использованы клеточные линии HEK293 и Vero, предоставленные Институтом синтетической биологии и геномной инженерии ФГБУ «ЦСП» ФМБА России.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Тузова Ирина Игоревна Ituzova@cspfmbs.ru

Статья поступила: 04.06.2025 **После доработки:** 03.09.2025 **Принята к публикации:** 01.10.2025 **Online first:** 27.10.2025

INTRODUCTION

The majority of modern biotechnological processes rely on the use of adherent cell lines, such as MRC5, Vero, MDCK, and HEK293, as substrates for virus accumulation [1].

The Vero cell line was derived in 1962 from kidney cells of a female African green monkey. This is a continuously cultivable cell line suitable for long-term maintenance without acquiring tumorigenic properties [2]. Due to the low expression of interferon and biosafety, this was the first cell line among those approved by the World Health Organization for use as a substrate for vaccines, continuing to maintain its significance up to the present [3].

The HEK293 cell line represents immortalized human embryonic kidney cells transformed by adenovirus serotype 5 genes. This transformation made HEK293 cells particularly useful as a substrate for accumulating recombinant proteins that require post-translational modifications. The presence of integrated *E1A* and *E1B* genes on chromosome 19 allows for the generation of recombinant adeno-associated particles. Unlike the Vero cell line, HEK293 exhibits genomic instability [4, 5].

Economic and social factors, such as the SARS-CoV-2 pandemic, have stimulated the demand for large volumes of antigen-accumulation substrate biomass and new preventive biopreparations [6, 7].

One of the main challenges in scaling up monolayer cell lines for industrial purposes consists in the need to increase the amount of harvested cell biomass [8]. Four methods for growing large volumes of cell cultures currently exist: static [9], dynamic [10], suspension-based [10, 11], and the use of micro- and macronutrient carriers [10, 12].

In comparison with other cultivation methods, roller (dynamic) cultivation offers a number of advantages:

- economical use of nutrients and reagents with higher titers of virus-containing material per unit volume compared to cultivation in culture flasks and cell factories. The increase in growth surface area by several times is achieved through ribbed vessel walls, while maintaining a compact size of the culture flask and minimal increase in medium volume. The manufacturers recommend filling a roller bottle (1900 cm²) with 300–400 mL of nutrient medium. For comparison, a smooth-wall roller flask of similar volume (area 850 cm²) contains up to 250 mL; therefore, more than doubling the surface area increases the medium volume by less than 1.5 times;
- lower cost of required equipment for biomass production, especially when using roller installations that fit into a standard CO₂ incubator or thermostat, compared to systems employing bioreactors and micro-nutrients;
- comparable number of operations performed by personnel when working with rollers and when working with culture flasks, yet providing for a larger growth surface area [13].

Although rotation frequency is one of the key factors affecting cell adhesion and monolayer formation [14], studies on this topic are scarce. When developing a methodology for rabies virus cultivation on Vero cell culture using roller bottles, the study [15] indicated an indirect relationship between rotation frequency and the quality of cell adhesion to the growth surface. This work evaluated changes in cell morphology during cultivation stages relying solely on proliferation index measurements. This study provided an assessment of

relationships between rotation frequency and the final cell number and division intensity [15]. For the HEK293 cell line, a specific cultivation protocol was mentioned: starting at 0.25 rpm followed by switching to 1 rpm for transfection with adeno-associated virus. At the same time, the authors offered no justifications for altering the rotation frequency range [16].

It should be noted that information on optimal rotation frequency rates during cultivation, their influence on cell adhesion during initial growth stages, as well as on increasing cell biomass growth and ultimate cell yield, is rather limited.

In this work, our objective was to optimize the initial stage of roller cultivation for Vero and HEK293 cell lines, taking into account the effects of roller bottle rotation frequency on cell adhesion during seeding and monolayer formation.

MATERIALS AND METHODS

In this study, HEK293 and Vero cell lines provided by the Institute of Synthetic Biology and Genetic Engineering of Centre for Strategic Planning of FMBA of Russia were used. Roller bottles with a growth surface area of 2125 cm² (maximum capacity of 2300 mL, diameter of 122 mm, ribbed walls, TC-treated polystyrene produced by Greiner) and culture flasks with an area of 175 cm² (TC-treated polystyrene, ServiceBio, China) were used as vessels for cultivation.

For the HEK293 cell line, Dulbecco's Modified Eagle Medium (ServiceBio, China) was used as the nutrient medium, supplemented with 10 mL of penicillin-streptomycin antibiotic solution (PanEko, Russia) per liter of ready-to-use medium, 1% v/v GlutaMAX solution (Gibco, UK), and 10% fetal bovine serum (Capricorn, UK).

For the Vero cell line, Earle's salt-modified medium (PanEko, Russia) was used, supplemented with 1% gentamicin sulfate (PanEko, Russia), 146 mg glutamine (PanEko, Russia), and 7.5% Embryo Replacement FetalClone III (HyClone, USA).

Initial seeding concentrations for both studied cell lines, determined according to recommended data in the cell culture passports, were identical and comprised 4×10^4 cells/cm² or 2.13×10^5 cells/mL. A cell suspension containing $85 \times 10^6 \pm 10\%$ cells was added to each roller bottle. The total volume of complete nutrient medium in the roller bottle was 400 mL (working volume according to manufacturer recommendations). Cultivation was carried out in a CO₂ incubator D180 (RWD) using a Centrol Mid roller assembly (Wiggins) at constant rotational speeds of 0.2, 0.3, 0.4, 0.5, and 0.6 rpm. These ranges were selected based on previously conducted studies on cultivating various cell lines in roller bottles [15, 16]. The presence of adherent cells, their morphology, and monolayer formation were assessed using microscopes, models TC5600 and TC5400 (Meiji Techno, Japan) at magnifications of $\times 10$ objective lens and $\times 10$ ocular lens.

The final cell count was determined using a C200FL cell counter and viability analyzer (Life Science, China). Prior to counting, the cells were dissociated with a 0.25% trypsin solution (PanEko, Russia) and resuspended in the appropriate nutrient medium described earlier for each cell culture. A sample of the cell suspension was diluted with 0.4% trypan blue dye (AbiDye, Russia) in a ratio of 1:1. Counting was repeated three times, and the average concentration of viable cells was calculated.

RESULTS AND DISCUSSION

When cultivating the Vero cell line at roller bottle rotation frequencies ranging 0.2–0.5 rpm, on day 1, cell adhesion was observed in all investigated roller bottles, with more than 90% of the seeded cells attached. At a rotation frequency of 0.6 rpm, fewer cells adhered to the plastic growth surface, leading to a lower-quality monolayer characterized by heterogeneity and inability to produce viral material. The quality criteria included the presence of a complete monolayer of cells without pronounced granularity under bright-field microscopy, absence of defects on the monolayer surface, and multi-layer cell growth filling the growth space. This high adhesive capacity of cells is attributed to an elevated integrin expression associated with tissue origin characteristics, since epithelial cells tightly arrange themselves together, forming layers.

Cells retained their characteristic morphology consistent with the cell line passport image. Although a tendency towards monolayer formation was observed at rotation frequencies of 0.2–0.5 rpm, on days 2 and 3 of cultivation, at a rotation frequency of 0.2 rpm, a notable aggregation of cells appeared on the monolayer surface. At rotation frequencies of 0.3–0.4 rpm, the number of cell aggregates decreased proportionately with an increase in rotation frequency. At 0.5 rpm, we observed the absence of cell aggregates on the surface, which were floating within the medium bulk. This phenomenon suggests that as an increase in rotation frequency (faster alternating cycles of feeding and breathing) creates more favorable conditions for cell growth. However, at rotation rates exceeding 0.5 rpm, the flow of culture medium induced by gravity and roller motion might disturb cell aggregates, causing them to detach from the surface.

For a greater visual clarity, photographs of Vero cells at a roller installation rotation frequency of 0.5 rpm were taken, illustrating the process of monolayer formation during cultivation (Figure 1). The color background differs in the photos due to different settings of the LED light source (built-in filter block) of the microscope used for photography, as well as changes in the color of phenol red in the nutrient medium caused by acid pH.

Figure 1 presents an image of cells 5 h after seeding, depicting their massive adhesion to the plastic growth surface and their typical polygonal morphology.

Figure 2 illustrates the process of monolayer formation during cultivation. We observed gradual filling of the growth space by cells and tightening of intercellular contacts due to limited growth space. The ribbed roller surface caused blurring closer to the edges of the field of view; as a result, capturing the entire developing monolayer in a single frame was not possible.

Following three days of cultivation, a dense monolayer with a confluence greater than 90% was obtained (Fig. 3), with no signs of cell aggregate clusters or degradation of the monolayer observed.

Thus, a roller bottle rotation frequency of 0.4–0.5 rpm ensured normal cell culture growth, since monolayer formation was associated with the least formation of cell aggregates. Cells attached and grew more evenly, and the forming monolayer did not deform. According to the literature data [15], cells indeed adhere better at a rotation frequency of 0.4–0.5 rpm. Therefore, after the onset of monolayer formation, increasing the rotation frequency is unnecessary. When culturing the Vero cell line within a pH range of 7.0–7.4, no significant differences

in cell adhesion to the growth surface were observed. In addition, temperature fluctuations within $\pm 2^{\circ}\text{C}$ of the standard temperature (37°C) during cultivation of the Vero cell line had no significant effect on cell adhesion.

The use of the selected optimal rotation frequency of the roller bottle during cultivation resulted in a cell biomass output reaching $386 \times 10^6 \pm 10\%$ cells per roller bottle.

For the HEK293 cell line, adhesion was observed at rotation frequencies ranging 0.1–0.4 rpm, indicating its lower adhesion capacity compared to the Vero cell line and its greater sensitivity to external mechanical disturbances due to genetic peculiarities and different tissue origins. At a rotation frequency of 0.2 rpm, the cells exhibited typical morphology: a flattened, slightly elongated shape with visible protrusions (Figure 4).

When culturing HEK293 cells for 6 h at a roller bottle rotation frequency of 0.4 rpm, the cells displayed atypical morphology (Figure 5). Despite attaching to the growth surface, their shape remained round, and upon subsequent division, small punctuate cell clusters formed. This phenomenon is presumably linked to strong mechanical impacts exerted by the culture medium, influenced by gravitational forces during roller rotation, which affect the cell membrane and destabilize connections between surface structures and chemical groups of the culture plastic [17, 18].

Meanwhile, when culturing HEK293 cells for 5 h at a roller bottle rotation frequency of 0.5 rpm, less than 50% of the seeded cells adhered, with most retaining a round shape similar to cells grown at rotation frequencies above 0.2 rpm (Figure 6). This is similarly explained by the strong influence of external mechanical forces during roller bottle rotation on cell shape and their ability to adhere to the growth surface. Upon increasing the rotation frequency to 0.6 rpm, no cell adhesion to the surface was observed.

Given that a roller bottle rotation frequency of 0.2 rpm provided the strongest cell adhesion to the growth

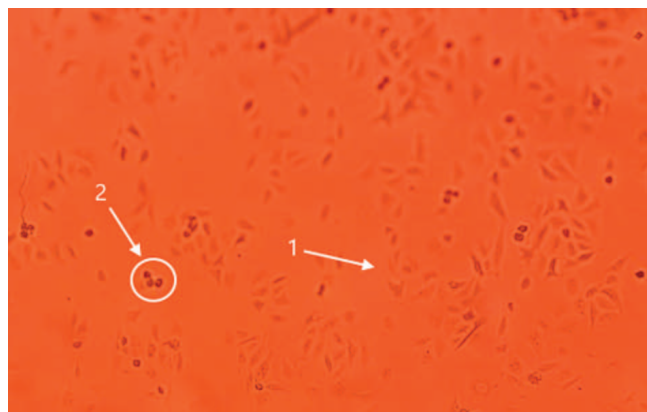


Photo taken by the authors

Fig. 1. Adhesion of Vero cells after 5 h of cultivation at a roller bottle rotation frequency of 0.5 rpm: 1 — cells attached to the surface; 2 — floating cells; magn. $\times 100$

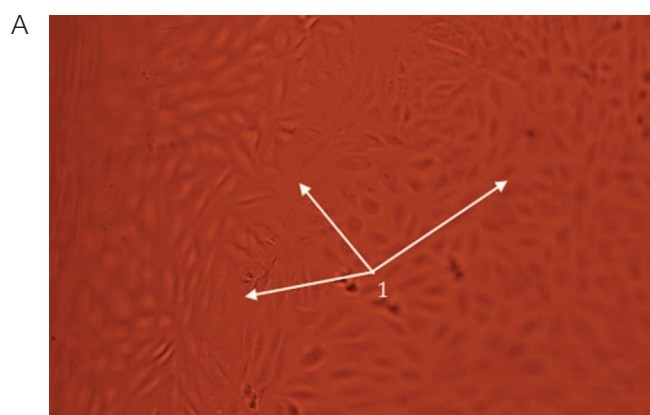


Photo taken by the authors

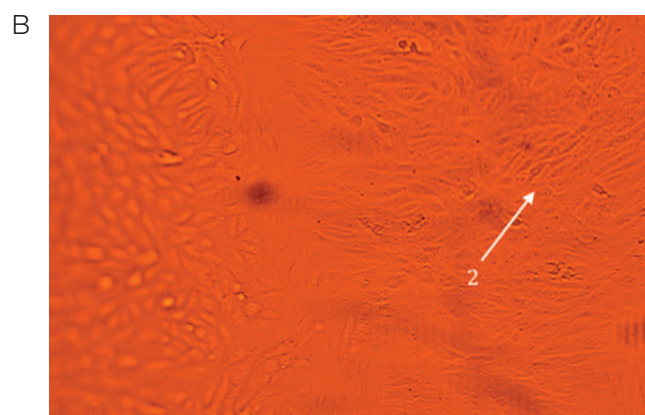


Fig. 2. Formation of Vero cell line monolayer on day 1 (A) and day 2 (B) of cultivation at a roller bottle rotation frequency of 0.5 rpm: 1 — areas of plastic growth surface; 2 — tight cell-cell contacts; magn. $\times 100$

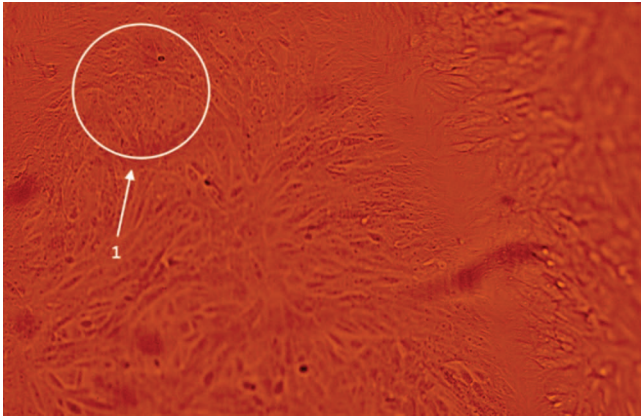


Photo taken by the authors

Fig. 3. Monolayer formed by Vero cell line after three days of cultivation at a roller bottle rotation frequency of 0.5 rpm: 1 — dense cell monolayer; magn. $\times 100$

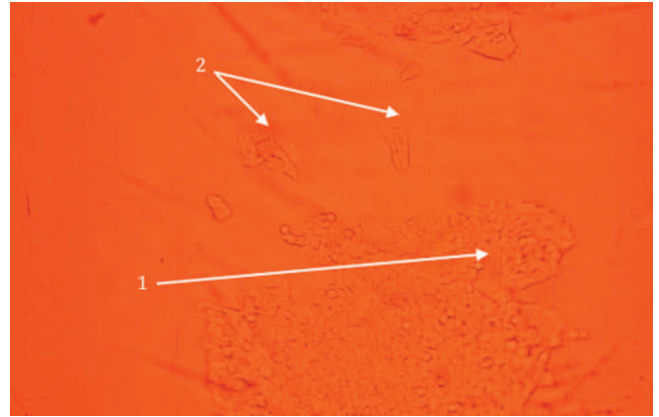


Photo taken by the authors

Fig. 4. Morphology of HEK293 cell line after 8 h of cultivation at a roller bottle rotation frequency of 0.2 rpm: 1 — cells attached to the growth surface; 2 — cell extensions; magn. $\times 100$

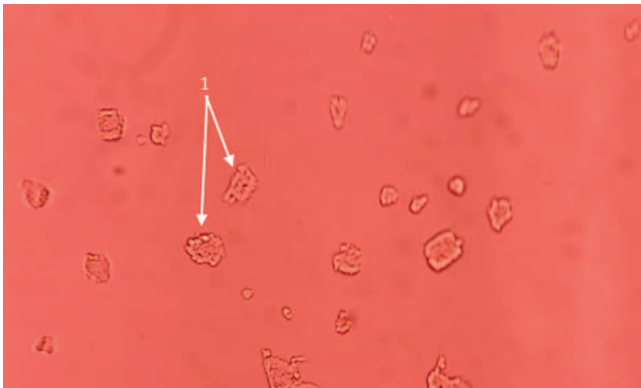


Photo taken by the authors

Fig. 5. Morphology of HEK293 cell line after 6 h of cultivation at a roller bottle rotation frequency of 0.4 rpm: 1 — round clusters of growing cells; magn. $\times 100$

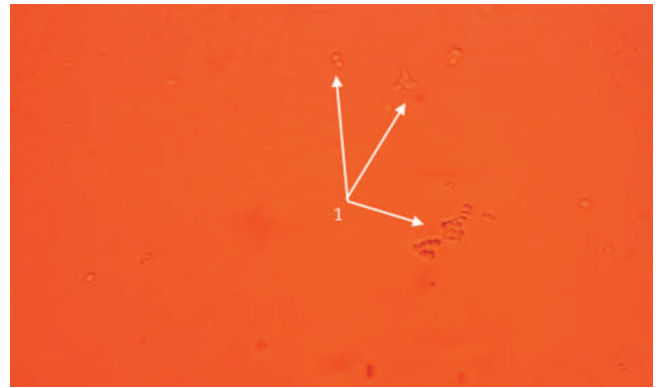


Photo taken by the authors

Fig. 6. Morphology of HEK293 cell line after 5 h of cultivation at a rotation frequency of 0.5 rpm: 1 — attached cells; magn. $\times 100$

surface while preserving cell morphology, monolayer formation was tracked under these conditions.

Upon culturing the HEK293 cell line at $37 \pm 2^\circ\text{C}$ and a rotation frequency of 0.2 rpm for 1 and 2 days, a tendency toward monolayer formation was observed (Figure 7). Despite active growth processes, a complete monolayer did not form on the remaining surface of the culture plastic; instead, multilayer cell growth occurred.

In the photographs, the color background varies due to changes in the color of phenol red in the nutrient medium when pH becomes more acidic. Since the roller surface was ribbed, causing blurred focus in the vicinity of the field-of-view edges, capturing the entire developing monolayer in a single frame was impossible.

By day 3, at a constant roller rotation frequency of 0.2 rpm, no complete monolayer formation had been

observed; simultaneously, areas not occupied by cell biomass were seen. Individual regions showed foci of multilayer cell growth with sharply demarcated boundaries between the monolayer and the plastic growth surface. Several detached floating cells in the nutrient medium were noted; the corresponding data is presented in Figure 8.

Therefore, after complete cell adherence to the plastic growth surface, the rotation frequency of the roller device was increased to 0.5 rpm to accelerate the transition between the feeding phase and the respiration phase. During slower transitions toward the respiration phase, cells might dry out and die later, leading to defects in the monolayer. In the sites with died cells, the absence of a direct physical contact between the cells leads to reactivated growth and mitotic processes in the cells. The most optimal

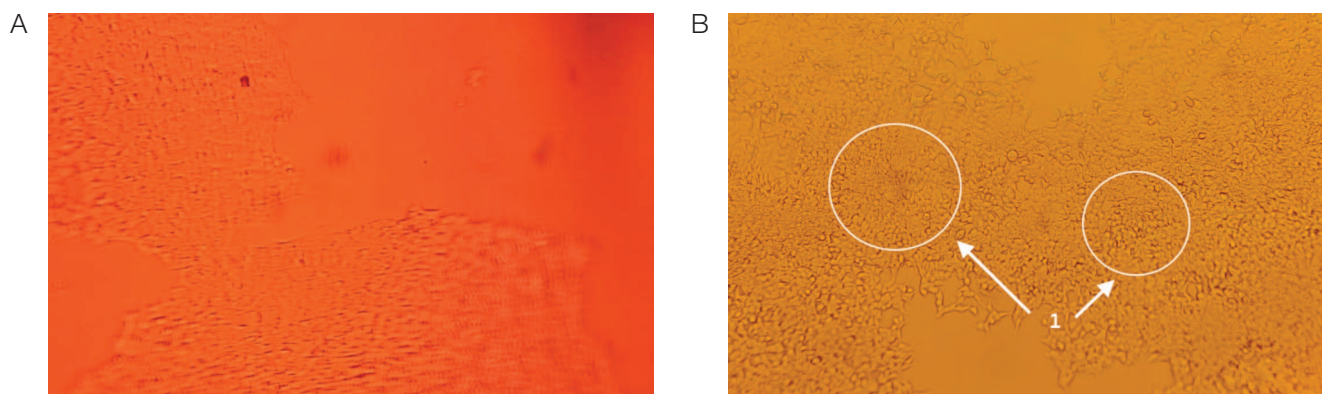


Photo taken by the authors

Fig. 7. Formation of HEK293 cell line monolayer during cultivation on day 1 (A) and day 2 (B) at a rotation frequency of 0.2 rpm: 1 — sections showing a tendency towards multilayer cell culture growth; magn. $\times 100$

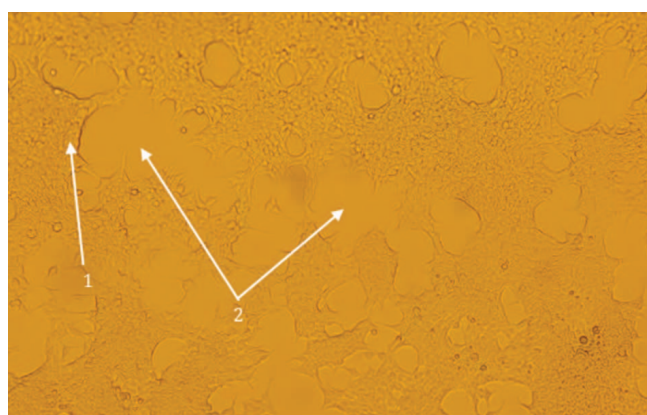


Photo taken by the authors

Fig. 8. Disruption of HEK293 cell line monolayer formation during 3-day cultivation at a roller bottle rotation frequency of 0.2 rpm: 1 — multilayer cell growth; 2 — plastic growth surface sections; magn. $\times 100$

rotation frequency for cell growth was established to be 0.5 rpm.

Additionally, we determined that, on day 2 of cultivation after cell seeding, the optimal increase in rotation frequency should not exceed 0.5 rpm; otherwise, there is an increased risk of deformation of the forming monolayer due to intense mechanical stress arising from the rotation of the roller apparatus and gravitational movement of the nutrient medium. The data obtained suggest that when culturing the HEK293 cell line, the rotation speed of the roller device should be changed to obtain a homogeneous monolayer.

Cultivation under the conditions proposed in this work, i.e., at $37 \pm 2^\circ\text{C}$ during day 1 at a rotation frequency of 0.2 rpm followed by an increase to 0.5 rpm on day 2, yielded a complete, high-quality monolayer (as depicted in Fig. 9). The monolayer consists of cells exhibiting a characteristic flattened shape upon attachment to the growth surface, with

a tendency toward the formation of large cell growth areas.

During the first day of cultivation at a roller bottle rotation frequency of 0.2 rpm, cells adhered to the growth surface, further forming growth areas of the cell culture (Figure 9A). Following the change in rotation frequency to 0.5 rpm, active cell growth was observed across the entire growth surface (Figure 9B) without areas of multilayer cell growth or sharp boundaries between the cell clusters and plastic.

By day 3 of cultivation at a rotation frequency of 0.5 rpm, a dense monolayer had formed, visually homogeneous, without focal points of multilayer cell growth or detached cells from the surface (Figure 10).

Our analysis of the cultivation conditions for the HEK293 cell line showed that pH values in the range of 7.0–7.4 and temperature variations within $\pm 2^\circ\text{C}$ of the standard range did not have a significant impact on cell adhesion to the growth surface. Using the proposed methodology with an increase in roller bottle rotation frequency on day 2 of cultivation, the yield of cell biomass reached $825 \times 10^6 \pm 10\%$ cells per roller by day 3 of cultivation.

Our results agree well with the data obtained by other researchers and illustrate a specific case of applying the cell lines under study. When selecting equipment for cultivation of the HEK293 cell line and lines with similar attachment features to the growth surface, it is advisable to consider the minimum rotation frequency of rollers in the roller assembly.

Future studies into cell adhesion to the growth surface for other cell lines will improve the efficiency of obtaining cell biomass as a substrate for accumulation and production of virus-containing materials.

CONCLUSION

As a result of our experimental research, the initial stage of roller cultivation for two cell lines — HEK293 and

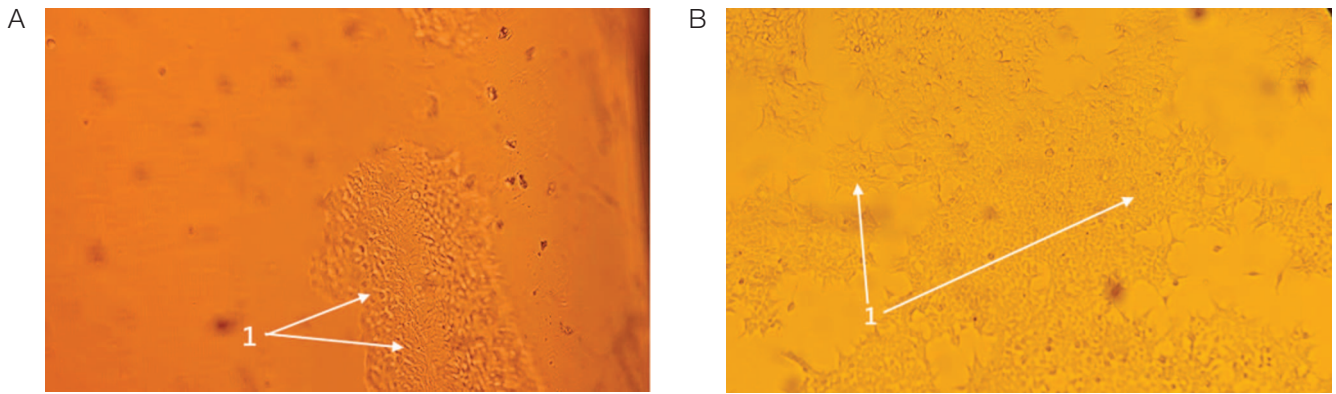


Photo taken by the authors

Fig. 9. Formation of HEK293 cell line monolayer during cultivation for: A — 1 day (rotation frequency 0.2 rpm); B — 2 days (rotation frequency 0.5 rpm); 1 — areas of cell culture growth with a tendency towards homogeneous monolayer formation; magn. $\times 100$

Vero — was optimized. The optimal rotation frequency for the Vero cell line was found to be 0.4–0.5 rpm throughout the entire cultivation period. In order to form a high-quality monolayer of HEK293, the initial rotation frequency should be 0.2 rpm, followed by an increase to 0.5 rpm.

The difference in rotation frequency for these two cell lines is explained by their tissue-specific characteristics. The Vero cell line demonstrates a higher degree of adhesion to the growth surface and resistance to external mechanical disturbances, compared to the HEK293 cell line.

The data obtained have practical significance for scaling up processes of cell and viral biomass production in monolayer cultivation, thus presenting interest to specialists involved in developing experimental viral preparations using adherent cell lines.

References

- Shen CF, Guilbault C, Li X, Elahi SM, Ansorge S, Kamen A, et al. Development of suspension adapted Vero cell culture process technology for production of viral vaccines. *Vaccine*. 2019;37(47):6996–7002. <https://doi.org/10.1016/j.vaccine.2019.07.003>
- Sene M-A, Xia Y, Kamen AA. Overview of recent advances in Vero cells genomic characterization and engineering for high-throughput vaccine manufacturing. *Clinical and Translational Discovery*. 2022;2(2):1–6. <https://doi.org/10.1002/ctd2.40>
- Kiesslich S, Kamen A. Vero cell upstream bioprocess development for the production of viral vectors and vaccines. *Biotechnology Advances*. 2020;44:1–9. <https://doi.org/10.1016/j.biotechadv.2020.107608>
- Malm M, Saghaleyni R, Lundqvist M, Giudici M, Chotteau V, Field R, et al. Evolution from adherent to suspension: systems biology of HEK293 cell line development. *Scientific Reports*. 2020;10:18996. <https://doi.org/10.1038/s41598-020-76137-8>
- Tan E, Chin CSH, Lim ZFS, Ng SK. HEK293 Cell Line as a Platform to Produce Recombinant Proteins and Viral Vectors. *Frontiers in Bioengineering and Biotechnology*. 2021;9:796991. <https://doi.org/10.3389/fbioe.2021.796991>
- Morozov AN, Yakhin IR, Stratonova NV, Kutsir MV, Poteryaev DA, Khamitov RA. An experience of scaling and intensifying the industrial production of the Gam-COVID-Vac vector adenovirus vaccine in the limiting conditions of the pandemic. *Biological Products. Prevention, Diagnosis, Treatment*. 2022;22(4):382–91 (In Russ.). <https://doi.org/10.30895/2221-996X-2022-22-4-382-391>
- Ishmukhametov AA. Fundamental and applied sciences, technology, and immunobiological products. *Herald of the Russian Academy of Sciences*. 2022;92(8):717–21 (In Russ.). <https://doi.org/10.31857/S0869587322080059>
- Babak VA, Lomako YuV, Gusev AA, Chaplygo KEH, Puntus IA, Filipkova AE. Optimal cultivation conditions for the BHK-21 cell line (c-13). *Transactions of the educational establishment "Vitebsk the Order of "the Badge of Honor" State Academy of Veterinary Medicine*. 2011;47(2–1):7–11 (In Russ.). EDN: SHRRRD
- Ryabova EI, Derkaev AA, Esmagambetov IB, Shcheblyakov DV, Dovgiy MA, Byrikhina DV, et al. Comparison of different technologies for producing recombinant adeno-associat-

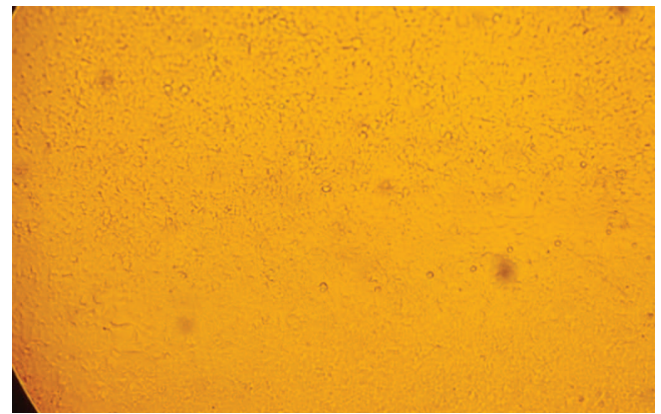


Photo taken by the authors

Fig. 10. Homogeneous monolayer formed by HEK293 cell line after three days of cultivation at 0.2 rpm, followed by a change in rotation frequency to 0.5 rpm on day 2 of cultivation: magn. $\times 100$

- ed virus on a laboratory scale. *BIOPreparations. Prevention, Diagnosis, Treatment*. 2021;21(4):266–78 (In Russ.).
<https://doi.org/10.30895/2221-996X-2021-21-4-266-278>
10. Bellani CF, Ajeian J, Duffy L, Miotto M, Groenewegen L, Connon CJ. Scale-Up Technologies for the Manufacture of Adherent Cells. *Frontiers in Nutrition*. 2020;7:575146.
<https://doi.org/10.3389/fnut.2020.575146>
 11. Sedova ES, Shcherbinin DN, Bandelyuk AS, Verkhovskaya LV, Viskova NYu, Avdonina ED, et al. Method for obtaining recombinant antibodies produced by a cell line transduced with recombinant adenoviruses. *Fine Chemical Technologies*. 2023;18(1):48–64 (In Russ.).
<https://doi.org/10.32362/2410-6593-2023-18-1-48-64>
 12. Yang J, Guertin P, Jia G, Lv Z, Yang H, Ju D. Large-scale microcarrier culture of HEK293T cells and Vero cells in single-use bioreactors. *Applied and Industrial Microbiology*. 2019;9(70):1–14.
<https://doi.org/10.1186/s13568-019-0794-5>
 13. Reshetnikova OV. Biotechnology of virus cultivation. *Current Issues in the Theory and Practice of Modern Biotechnology*. 2015:155–61 (In Russ.).
EDN: [XDGYLH](https://elibrary.ru/xdgylh)
 14. Alexander MH. *In vitro expansion of postpartum-derived cells in roller bottles*. Patent of United States No. 8741638B2; 2014.
 15. Generalov SV, Abramova EG, Matveeva ZhV, Zhulidov IM, Savitskaya LV, Lobovikova OA. Optimization of specifications for scaled-up fixed rabies virus cultivation ("Moscow 3253" strain) in Vero cell culture. *Problems of Particularly Dangerous Infections*. 2014;2:101–3 (In Russ.).
<https://doi.org/10.21055/0370-1069-2014-2-101-103>
 16. Liu YL, Wagner K, Robinson N, Sabatino D, Margaritis P, Xiao W, et al. Optimized Production of High-Titer Recombinant Adeno-Associated Virus in Roller Bottles. *BioTechniques*. 2003;34(1):184–9.
<https://doi.org/10.2144/03341dd07>
 17. Chisti Y. Hydrodynamic Damage to Animal Cells. *Critical Reviews in Biotechnology*. 2001;21(2):67–110.
<https://doi.org/10.1080/20013891081692>
 18. Chang HY, Kao WL, You YW, Chu YH, Chu KJ, Chen PJ, et al. Effect of surface potential on epithelial cell adhesion, proliferation and morphology. *Colloids and Surfaces B: Biointerfaces*. 2016;141:179–86.
<https://doi.org/10.1016/j.colsurfb.2016.01.049>

Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. The greatest contributions are as follows: Irina I. Tuzova — conducting the experimental part of the study, drafting a manuscript and its revision; Tatyana I. Chirkina — analyzing and interpreting the data obtained; Igor A. Churkin — conceptualizing and directing the study; Anastasia N. Lyakh — selecting materials and methods for the study; Kirill M. Mefed — choosing the direction of the experimental work and approving the final version of the article; Vladimir A. Maximov — administering the project.

AUTHORS

Irina I. Tuzova

ITuzova@cspfmmba.ru

Tatyana I. Chirkina

TChirkina@cspfmmba.ru

Igor A. Churkin, Cand. Sci. (Biol.)

ICHurkin@cspfmmba.ru

Anastasia N. Lyakh

ALyakh@cspfmmba.ru

Kirill M. Mefed, Cand. Sci. (Biol.)

<https://orcid.org/0000-0002-7335-1982>

KMefyod@cspfmmba.ru

Vladimir A. Maximov, Dr. Sci. (Med.)

vmaksimov@cspfmmba.ru

<https://doi.org/10.47183/mes.2025-384>



EVALUATION OF THE FREQUENCY OF ULTRA-SHORT AND ULTRA-LONG TELOMERES IN CHRONICALLY EXPOSED WOMEN

Yana V. Krivoshchapova[✉], Yulia R. Akhmadullina

Southern Urals Federal Research and Clinical Center for Medical Biophysics, Ozersk, Russia

Introduction. Telomere length is considered a potential biomarker for individual human radiosensitivity and radioresistance. Radiation exposure can both increase and decrease the average telomere length in cells, while the lengths of individual telomeres vary widely. The assessment of ultra-short and ultra-long telomere frequency may indicate alterations in the replicative potential of cells and radiation-induced genomic instability.

Objective. To investigate the relative telomere length using the Q-FISH method in exposed individuals and to identify the proportion of ultra-short and ultra-long chromosomal telomeric regions in this cohort.

Materials and methods. The study involved 43 volunteer donors (women) from different age groups (21–28; 60–67; and 71–83 years). At stage I, an investigation of the dose-dependence of relative telomere length was performed in the control group. The donors were divided into groups: younger ($n = 4$) — non-exposed women aged 21–28 years; middle-aged ($n = 12$) — women aged 60–67 years; older ($n = 5$) — women aged 71–83 years. At stage II, the reference for average telomere length was established using donors ($n = 5$) from the older age group. At stage III, considering the established reference values, telomere length was studied in exposed individuals ($n = 22$), including analysis based on age and bone marrow dose. Cytogenetic preparations were obtained according to a protocol that includes cell culturing to the metaphase stage, hypotonic treatment, fixation of metaphase plates, and chromosome slide preparation. Telomeres were fluorescently stained using probes (DAKO, Denmark) in accordance with the manufacturer's protocol. Standard methods of descriptive and comparative statistics were used.

Results. In exposed individuals, the median telomere length was statistically significantly higher than that in the comparison group (10.3% vs. 5.8%, $p = 0.0001$). Concurrently, this group exhibited a reduced frequency of ultra-short telomeres (1.6% vs. 5%) and an increased frequency of ultra-long telomeres (19.5% vs. 5%, $p < 0.0001$). A case-control study confirmed this pattern for the individuals with medium and high bone marrow doses. A statistically significant decrease in median telomere length was observed in donors with a high bone marrow dose compared to those with medium doses (11.9% vs. 10.6%, $p = 0.0001$). An increase in the bone marrow dose led to an exponential decrease in the frequency of ultra-short telomeres ($R^2 = 0.23$, $p = 0.0036$).

Conclusions. A decrease in relative telomere length was observed in non-exposed individuals with an increase in age. In the group of young donors aged 20–28 years, the median telomere length was 31.0%, comprising 13.0% and 5.8% ($p = 0.0001$) in the 60–67 and 71–83 age groups, respectively. The reference range for ultra-short telomeres in the 71–83 year group was 0–0.7%, being 25.6% and above for ultra-long telomeres. In exposed individuals, the median telomere length was statistically significantly higher than in the comparison group ($p = 0.0001$). Exposed individuals exhibited a reduced frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres with respect to the comparison group ($p = 0.004$). A non-linear regression dependence of the frequency of ultra-short telomeres on bone marrow dose manifested in an exponential decrease in frequency with an increase in dose was noted.

Keywords: telomeres; telomere length; short telomeres; long telomeres; women; chronic radiation exposure; the Techa River

For citation: Krivoshchapova Ya.V., Akhmadullina Yu.R. Evaluation of the frequency of ultra-short and ultra-long telomeres in chronically exposed women. *Extreme Medicine*. 2026;28(1):69–78. <https://doi.org/10.47183/mes.2025-384>

Funding: the study was conducted within the framework of the state assignment of the Federal Medical and Biological Agency “Long-term cytogenetic effects of chronic radiation exposure in residents of the Southern Urals” (No. 388-03-2025-085, code: Cytogenetic Effects).

Compliance with ethical principles: the study was approved by the Ethics Committee of the Southern Urals Federal Research and Clinical Center for Medical Biophysics (Minutes No. 6, of 19.12.2022). Written informed consent was obtained from all participants prior to their inclusion in the study.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Yana V. Krivoshchapova Yana_ho@mail.ru

Received: 28 Aug. 2025 **Revised:** 1 Nov. 2025 **Accepted:** 12 Nov. 2025 **Online first:** 27 Dec. 2025

УДК 614.876:539.1.04:575.224.232

ОЦЕНКА ЧАСТОТЫ СВЕРХКОРОТКИХ И СВЕРХДЛИННЫХ ТЕЛОМЕР У ХРОНИЧЕСКИ ОБЛУЧЕННЫХ ЖЕНЩИН

Я.В. Кривошчапова[✉], Ю.Р. Ахмадуллина

Южно-Уральский федеральный научно-клинический центр медицинской биофизики, Озерск, Россия

Введение. Длина теломер рассматривается как потенциальный биомаркер индивидуальной радиочувствительности и радиорезистентности человека. Радиационное облучение может как увеличивать, так и уменьшать среднюю длину теломер в клетках, при этом показатели длин отдельных теломер варьируют в широком диапазоне. Оценка частоты сверхкоротких и сверхдлинных теломер может указывать на изменения репликативного потенциала клеток и на радиационно-индуцированные нарушения генома.

Цель. Изучить относительную длину теломер методом Q-FISH у облученных лиц, а также выявить у них долю сверхкоротких и сверхдлинных теломерных участков хромосом.

© Ya.V. Krivoshchapova, Yu.R. Akhmadullina, 2025

Материалы и методы. Исследование проведено с участием 43 доноров-добровольцев (женщин) различных возрастных групп (21–28, 60–67, 71–83 года). На первом этапе в контрольной группе проведено исследование зависимости относительной длины теломер от возраста. Доноры были разделены на группы: младшая ($n = 4$) — не подвергавшиеся облучению женщины в возрасте 21–28 лет; средняя ($n = 12$) — женщины в возрасте 60–67 лет; старшая ($n = 5$) — женщины в возрасте 71–83 года. На II этапе у доноров ($n = 5$) старшей возрастной группы был определен референс средней длины теломер. На III этапе, учитывая установленные референсные значения, изучали длину теломер у облученных лиц ($n = 22$), в том числе в зависимости от возраста и дозы облучения красного костного мозга (ККМ). Цитогенетические препараты получали согласно протоколу, который включает культивирование клеток до стадии метафазы, гипотоническую обработку, фиксацию метафазных пластинок и получение препаратов хромосом. Флуоресцентная окраска теломер проводилась зондами (ДАКО, Дания) в соответствии с протоколом производителя. В работе использовали стандартные методы описательной и сравнительной статистики.

Результаты. У облученных лиц медианная длина теломер статистически значимо выше, чем в группе сравнения (10,3% против 5,8%, $p = 0,0001$), при этом у них снижена частота сверхкоротких теломер (1,6% против 5%) и повышена частота сверхдлинных (19,5% против 5%, $p < 0,0001$). Исследование методом «случай — контроль» подтвердило данную закономерность для лиц со средней и высокой дозой облучения ККМ. Наблюдалось статистически значимое снижение медианной длины теломер в группе доноров с высокой дозой облучения ККМ относительно лиц со средними дозами (11,9% против 10,6%, $p = 0,0001$). С увеличением дозы облучения ККМ частота сверхкоротких теломер экспоненциально уменьшалась ($R^2 = 0,23$, $p = 0,0036$).

Выводы. Отмечено снижение относительной длины теломер у необлученных лиц с увеличением возраста, в группе молодых доноров 20–28 лет медианное значение длины теломер составило 31,0%, в группе 60–67 лет — 13,0%, в группе 71–83 года — 5,8% ($p = 0,0001$). Референсное значение сверхкоротких теломер для возрастной группы 71–83 года составило 0–0,7%, а сверхдлинных — от 25,6% и выше. У облученных лиц медианная длина теломер статистически значимо выше, чем в группе сравнения ($p = 0,0001$). У облученных лиц снижена частота сверхкоротких теломер и выше частота сверхдлинных теломер относительно группы сравнения ($p = 0,004$). Отмечена нелинейная регрессионная зависимость частоты сверхкоротких теломер от дозы облучения ККМ: с увеличением дозы облучения частота экспоненциально уменьшается.

Ключевые слова: теломеры; длина теломер; короткие теломеры; длинные теломеры; женщины; хроническое радиационное воздействие; река Теча

Для цитирования: Кривошапова Я.В., Ахмадуллина Ю.Р. Оценка частоты сверхкоротких и сверхдлинных теломер у хронически облученных женщин. *Медицина экстремальных ситуаций*. 2026;28(1):69–78. <https://doi.org/10.47183/mes.2025-384>

Финансирование: работа выполнена в рамках государственного задания Федерального медико-биологического агентства «Отдаленные цитогенетические эффекты хронического облучения у жителей Южного Урала» (№ 388-03-2025-085, шифр: «Цитогенетические эффекты»).

Соответствие принципам этики: исследование одобрено комитетом по этике ФГБУН ЮУрФНКЦ МБ ФМБА России (протокол № 10 от 19.12.2022). Всеми участниками подписано добровольное информированное согласие на участие в исследовании.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Кривошапова Яна Владимировна Yana_ho@mail.ru

Статья поступила: 28.08.2025 **После доработки:** 01.11.2025 **Принята к публикации:** 12.11.2025 **Online first:** 27.12.2025

INTRODUCTION

According to current understanding, telomeres are essential terminal regions of chromosomes that perform a multitude of functions, with the primary one being the maintenance of genomic integrity. It has been noted that telomere length varies widely not only among individuals but even within a single cell and across chromosomal pairs, which can be explained by uneven telomere shortening [1, 2]. This phenomenon of telomere length distribution along the arms of individual chromosomes is referred to as the “chromosomal telomere profile” [3]. The telomere profile can serve as a potential biomarker for predicting the risk of certain diseases and for assessing the impact of adverse external factors, including ionizing radiation [4]. Consequently, research aimed at studying the influence of radiation exposure on telomere length is highly relevant. Exposure of individuals due to atomic bombings in Hiroshima and Nagasaki to doses conventionally categorized as low (5–700 mSv) and high (over 700 mSv) led to telomere shortening; these alterations persisted even in the long term (50–68 years post-exposure) in the high-dose group [5]. Conversely, some studies have reported an increase in telomere length in

exposed individuals, which has been associated with elevated telomerase expression [6].

An assessment of relative telomere length in nuclear industry workers exposed to internal alpha (0.05 Gy; 0.05–0.1 Gy; > 0.1 Gy) and external gamma (1 Gy; 0.1–1.5 Gy; > 1.5 Gy) radiation found this parameter to decrease only after low-dose exposure, for both external gamma radiation (at doses < 1 Gy) and internal alpha radiation (at doses < 0.05–0.1 Gy). At higher doses, this parameter did not differ from the values in the non-exposed control group [7]. A study involving individuals chronically exposed at a wide dose range (0–4.7 Gy) revealed a decrease in relative telomere length under chronic exposure (0.6–4.7 Gy) compared to the control group for specific chromosomal arms of meta- and acrocentric chromosomes. No monotonic decrease in telomere length was observed for chromosomes within a single cell or across different arms of the same chromosome [1]. This indicates that telomere length is a dynamic structure determined not merely by chronological age but rather by the combined influence of numerous endogenous and exogenous factors.

At present, researchers are increasingly addressing the relationship between telomere length and cell and

body radiosensitivity [8]. For instance, certain diseases characterized by clinical hyper-radiosensitivity (ataxia-telangiectasia, Fragile X syndrome, Fanconi anemia) were shown to be associated with impairments in telomere maintenance [9]. Investigations into the radiosensitivity of human fibroblasts exposed to X-ray radiation in vitro demonstrated that cell clones that retained viability after a 4 Gy irradiation dose exhibited increased telomere length compared to non-irradiated clones. The authors advanced a hypothesis that cells with longer telomeres are more radioresistant, and that radiosensitivity correlates with telomere loss rather than with their average length [4].

A number of studies have shown that the quantity of ultra-short telomeres, rather than their average length, serves as the most informative predictive marker for the development of certain diseases [10]. For instance, an association between the frequency of ultra-short telomeres and various human pathologies, such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, has been established [11].

All existing methods for studying chromosome telomeres can be broadly distinguished into two groups: molecular and molecular-cytogenetic. The molecular-cytogenetic method of quantitative fluorescence in-situ hybridization (Q-FISH) on metaphase chromosomes is the most suitable approach for the individual assessment of telomeric regions. This method employs fluorescently labeled peptide nucleic acids (PNA), which specifically hybridize with denatured telomeric DNA. Using the Q-FISH method, telomeric regions can be examined in each chromosome in every cell, since chromosome karyotyping is performed during the analysis [1].

In this study, we focus on telomere length in individuals chronically exposed to a wide dose range due to residence in radionuclide-contaminated areas, during long-term follow-up (60–65 years post-exposure). The radiation exposure was combined: internal, i.e., due to the intake and accumulation of ^{89,90}Sr radionuclides in the body, and external — from γ-radiation. The critical organ for radiation exposure was the red bone marrow (RBM). Doses to the RBM were predominantly accumulated before 1960 [12].

Our aim was to investigate telomere length using the Q-FISH method in exposed individuals and to identify the proportion of ultra-short and ultra-long chromosomal telomeric regions in this cohort.

MATERIALS AND METHODS

Study design

The study involved 43 female donors from different age groups (21–28; 60–67; 71–83 years). The inclusion criteria were the absence of a history of autoimmune or oncological diseases, and chronic inflammatory diseases in the acute phase. Individuals taking cytostatics or antibiotics were excluded. Health status information for the exposed individuals was obtained from the Database “Chelovek” (Man); individual doses were calculated using TRDS-2016 in the Biophysics Laboratory. Information on cancer history in the examined individuals was provided by an Epidemiology Laboratory [13].

At the first stage, an investigation of the age-dependence of relative telomere length was conducted in a control group (donor characteristics are presented in Table 1). Three age groups of donors were formed:

- younger age group — 4 unexposed women aged 21–28 years (mean age 24 ± 2.9 years);
- middle-aged group — 12 donors aged 60–67 years (mean age 63.8 ± 1.8 years);
- older age group — 5 donors aged 71–83 years (mean age 76.4 ± 5 years).

Although donors from the older age group resided in radionuclide-contaminated areas, their accumulated bone marrow dose did not exceed 0.05 Gy over their lifetime. Consequently, hereafter (in stages II and III of the study), these donors will be referred to as non-exposed individuals or the comparison group.

At stage II, the reference for average telomere length was established in the comparison group. At stage III, taking into account the established reference values, telomere length was studied in exposed individuals, including analysis based on age and bone marrow dose.

With the purpose of assessing the influence of ionizing radiation on telomere length, two groups of exposed

Table 1. Characteristics of donors at the first study stage

Parameter	Age group of donors		
	younger age	middle-aged group	older age
Number of donors, N	4	12	5
Age, years, M (min–max)	24 (21–28)	63.8 (60–67)	76.4 (71–83)
Bone marrow dose, Gy	0	0–0.05	0–0.05
Number of telomere region measurements, abs.	4,331	22,311	9,732

Table compiled by the authors based on their original data

Note: age is presented as the mean value along with the maximum and minimum values.

donors were formed: those exposed to doses ranging 0.14–0.86 Gy (10 individuals aged 69–80 years) constituted the medium-dose exposure group; the high-dose exposure group comprised 12 individuals aged 71–84 years with bone marrow doses of 1.04–3.1 Gy (Table 2).

Furthermore, a case-control analysis was conducted to eliminate the influence of the age factor. To that end, potential controls for each donor (“case”) from the medium-dose exposure group were selected based on the criterion of matching the age at the time of examination. The bone marrow dose was considered the influencing factor. Four pairs of donors of similar age were formed. In each pair, one donor had an established accumulated bone marrow dose ranging 0.61–0.86 Gy, while the other donor in the pair either had not been accidentally exposed or had a dose of ≤ 0.05 Gy (Table 3).

For the case-control study within the high bone marrow dose range, five pairs of donors of similar age were selected. One donor in each pair had a dose exceeding

1 Gy, and the other donor either had not been accidentally exposed or had a dose of ≤ 0.05 Gy (Table 3).

Methodology for metaphase chromosome preparation

The subject of the study was the nuclear chromatin of T-lymphocytes from peripheral blood stimulated with phytohaemagglutinin (PHA). T-lymphocytes were chosen due to the ease of obtaining the source material and their sufficiently high concentration. T-lymphocytes do not divide in peripheral blood and reside in the G0 phase of the cell cycle, representing a naturally synchronized cell population in the body. Lymphocyte precursor cells are irradiated directly in the RBM.

Blood was drawn into vacuum plastic blood collection tubes containing lithium heparin.

The culture mixture was prepared from the following components: 2 mL of whole heparinized blood, 5 mL of RPMI-1640 medium (PanEco, Russia), 0.5 mL of

Table 2. Characteristics of exposed donors

Parameter	All exposed individuals	Medium doses 0.14–0.86 Gy	High doses 1.04–3.1 Gy
Age, years, M (min–max)	74.6 (69–84)	73.4 (69–80)	75.7 (71–84)
Number of donors, N	22	10	12
Bone marrow dose, Gy	0.14–3.1	0.14–0.86	1.04–3.1
Number of telomere region measurements, abs.	38,416	18,852	19,564

Table compiled by the authors based on their original data

Note: age is presented as the mean value along with the maximum and minimum values.

Table 3. Characteristics of donors in the case-control study

Pair No. in the case-control study	Donor age, years		Bone marrow dose, Gy		Number of measurements, abs.	
	case	control	case	control	case	control
Exposed to medium doses (0.14–0.86 Gy)						
1	80	80	0.64	0.003	1,748	1,456
2	74	75	0.8	0.05	3,810	1,458
3	72	73	0.86	0.008	3,208	2,630
4	71	71	0.61	0	1,918	2,744
Exposed to high doses (1.04–3.1 Gy)						
1	84	83	1.04	0.03	1,610	1,444
2	81	80	1.25	0.003	1,850	1,456
3	71	71	1.57	0	1,836	2,744
4	73	73	1.93	0.008	1,922	2,630
5	74	75	2.73	0.05	1,840	1,458

Table compiled by the authors based on their original data

selected fetal bovine serum (PAA Laboratories, Austria). PHA was added to a final concentration of 20 µg/mL (PanEco, Russia). Cells were cultured in a CO₂ incubator (SANYO MCO-18AIC, Japan) at 37.5 °C for 54 h. Three hours prior to the end of cultivation, colchicine was added to achieve the final concentration of 0.1 µg/mL (PanEco, Russia).

Subsequently, metaphase cells underwent hypotonic treatment, fixation of metaphase plates (using a 3:1 mixture of 95% medical-grade ethanol and glacial acetic acid), and chromosome slide preparation. For fluorescent staining using the Q-FISH method, slides were treated with RNase (100 ng/mL) and pepsin solutions, followed by denaturation of the probe and specimen DNA. Hybridization was performed in accordance with the manufacturer's protocol, which was supplied with the original reagent solutions.

To assess the length of chromosomal telomeric regions, DAKO probes (Denmark) were used. Centromeric probes (Metasystems; Germany) were applied to stain the centromeric region of chromosome 2, which served as a reference signal. Analysis of fluorescently stained specimens was performed using an AxioImager Z2 fluorescence microscope (Zeiss; Germany) equipped with DAPI and SpO (spectrum orange) filters.

The intensity of the recorded fluorescent signal was measured using the Isis specialized image analysis software. For each subject, 20–30 cells were analyzed. Results were obtained separately for the short (p-) and long (q-) arms of each of the 46 chromosomes. Telomere length was relative, being expressed as a percentage of the telomeric (T) signal length relative to the centromeric (C) signal length — (T/C%). The method for assessing telomeric region length is described in detail in [14].

Standard methods of descriptive statistics were used to process the data obtained, including calculation of the median, its 95% confidence interval (95% CI), and the 5th and 95th percentiles (%). Confidence intervals were calculated using a bootstrap procedure with $n = 1000$ resamples. Intergroup comparisons were performed using the non-parametric Mann–Whitney U test.

The threshold for ultra-short telomeres was defined as the value below which the lowest 5% of all telomere length measurements in the non-exposed group fall.

Similarly, the reference value for ultra-long telomeres was calculated as all values exceeding the 95th percentile of the measurement distribution. The obtained reference values were used for comparative analysis with data on the relative telomere length in individuals exposed at various dose ranges.

When comparing the frequency of ultra-short and ultra-long telomeres in the case-control study and against the reference value, the χ^2 test was used. Multivariate analysis was performed to determine the association of telomere length with age and dose. Spearman's correlation coefficient and regression analysis were employed to assess the relationship between the dose and the number of ultra-short and ultra-long telomeres per individual. Statistical analysis was conducted using the following software packages: Past 4.01 (Oyvind Hammer; UK), Statistica 10 (StatSoft, USA), and SigmaPlot (SYSTAT Software, USA).

RESULTS

During the study, data on telomere length measurements were obtained for three age groups of non-exposed donors (Table 4).

In the donor group aged 20–28 years, 4331 measurements of telomeric regions were performed. The minimum recorded telomere length was 0%, the maximum was 900.0%, and the median telomere length was 31.0%.

Simultaneously, in the middle-aged donor group (60–67 years), 22,311 measurements of telomeric regions were performed. The telomere length range (min–max) was 0–967.9%, and the median value was 13.0%. In the donor group aged 71–83 years, 9732 measurements were obtained. The minimum telomere length was 0%, the maximum was 141.2%, and the median was 5.8%.

The values for ultra-short telomeres in the younger donor group ranged 0–6.3%; for donors aged 60–67 and 71–83 years the values were 0–1.8 and 0–0.7%, respectively. Meanwhile, the ranges for ultra-long telomeres were 221.2–900.0% for donors aged 20–28 years, 105.9–967.9% for donors aged 60–67 years, and 25.6–141.2% for donors aged 71–83 years.

Table 4. Length of chromosomal telomeric regions in non-exposed donors by age (T/C %)

Relative length of chromosomal telomeric regions, T/C %	Age group of donors		
	younger age (21–28 y.o.)	middle-aged (60–67 y.o.)	older age (71–83 y.o.)
min–max values	0–900.0	0–967.9	0–141.2
Median	31.0*	13.0*	5.8*
[95% CI]	[28.5–32.6]	[12.8–13.3]	[5.6–5.9]
5–95% percentiles	6.3–221.2	1.8–105.9	0.7–25.6

Table compiled by the authors based on their original data

Note: * statistically significant differences between groups (Mann–Whitney U test), $p = 0,0001$; y.o. — years old

It was established that median telomere length decreased with an increase in donor age. Pairwise comparison of values in the studied control groups revealed statistically significant differences ($p = 0.0001$). Regression analysis demonstrated a statistically significant ($p < 0.001$; $R^2 = 0.07$), albeit extremely weak, linear dependence between relative telomere length and donor age:

$$y = 86.1 - 26.7 x,$$

(1)

y — relative telomere length, x — age of the examined individuals.

For the subsequent analysis of telomere length in exposed individuals, a reference value of 0.7% was adopted for ultra-short telomeres, corresponding to the lower bound of the 90% telomere length range in non-exposed donors aged 71–83 years. Ultra-long telomeres were defined using the upper bound of the 90% telomere length range — 25.6%.

Table 5 presents the telomere length measurement data for exposed donors. The comparison group consists of non-exposed donors aged 71–83 years.

It can be seen that the median telomere length for all exposed individuals was 10.3%, which is statistically significantly higher than that in the comparison group (5.8%; $p = 0.0001$). At the same time, the 90% interval of telomere length ranged 1.4–56.5%.

Considering the reference value for ultra-short telomeres, within the group of exposed donors (0.14–3.1 Gy), 616 telomere measurements were classified as ultra-short, constituting 1.6% of all measurements. Within the reference range for ultra-long telomeres, exposed donors had 7491 measurements, accounting for 19.5%. Thus, exposed individuals exhibited a lower number of ultra-short telomeres ($\chi^2 = 397.03$; $p < 0.0001$) and a higher number of ultra-long telomeres ($\chi^2 = 1184$; $p < 0.0001$) relative to the comparison group.

In the medium-dose exposure group (0.14–0.86 Gy), the median telomere length was 11.9% (min–max:

0–643.4%). For donors exposed to high doses, the median was 10.6% (min–max: 0–332.1%), which is statistically significantly lower than in the medium-dose group ($p = 0.0001$).

Relative to the reference values, the frequency of ultra-short telomeres in donors exposed to medium doses was 1.3%, and 1.4% in those exposed to high doses. The frequency of ultra-long telomeres was 24% in the medium-dose group and 19.5% in the high-dose group, which was statistically significantly different from the values in the comparison group ($p = 0.0001$). Thus, in exposed individuals with a high accumulated bone marrow dose, the frequency of ultra-short telomeres is similar to that in the medium-dose group. At the same time, in the former group, the number of ultra-long telomeres is higher.

The relationship between telomere length, age, and bone marrow dose was established by a two-factor analysis (general linear model). A statistically non-significant model where telomere length in the entire sample of exposed individuals did not depend on age or bone marrow dose was obtained.

Table 6 presents data on the pairwise comparison for the case-control analysis, where the “cases” are individuals with medium (0.14–0.86 Gy) and high (1.04–3.1 Gy) accumulated bone marrow doses.

In all examined pairs from the medium dose group, a statistically significant increase in telomere length was detected in exposed donors compared to their controls.

In the group of individuals exposed to high doses, a statistically significant increase in telomere length in exposed donors compared to their controls in four out of five pairs was found. In Pair No. 1, a statistically significant decrease in telomere length was observed in the exposed donor relative to their control. However, in this specific case-control pair, the frequency of ultra-short telomeres did not differ from the control values (0.8% vs. 0.7%; $\chi^2 = 0.03$; $p = 0.86$), while the frequency of ultra-long telomeres was significantly lower (1.4% vs. 2.9%; $\chi^2 = 8$; $p = 0.005$) (Table 7).

Table 5. Length of chromosomal telomeric regions in exposed donors (T/C %)

Relative length of chromosomal telomeric regions, T/C %	Groups of exposed donors		
	All exposed individuals	Medium doses (0.14–0.86 Gy)	High doses (1.04–3.1 Gy)
Min–max values	0–643.4	0–643.4	0–332.1
Median [95% CI]	10.3 [10.2–10.4]	11.9 [11.7–12.1]*	10.6 [10.4–10.8]
5–95% percentiles	1.4–56.5	1.6–61.7	1.4–57.7
Share of ultra-short telomeres, %	1.6	1.3	1.4
Share of ultra-long telomeres, %	19.5	24*	19.5

Table compiled by the authors based on their original data

Note: * — statistically significant difference from the high-dose exposure group, $p \leq 0.0001$.

Table 6. Pairwise comparison of telomeric region length in the case-control study for medium doses (0.14–0.86 Gy) and high doses (1.04–3.1 Gy)

Pair No. in the case- control study	Relative length of chromosomal telomeric regions, T/C %								Mann– Whitney <i>U</i> test
	Min–max		Median		5%		95%		
	case	control	case	control	case	control	case	control	
Medium doses (0.14–0.86 Gy)									
1	0–143.3	0–66.2	17.0	3.5	3.4	0.1	54.5	17.9	<i>p</i> = 0.0001
2	0–267.1	0–119.1	16.9	6.6	3.7	0.06	68.6	32.6	<i>p</i> = 0.0001
3	0.13–71.7	0–65.8	3.9	3.6	0.8	0.8	16.8	10.7	<i>p</i> = 0.0001
4	0–643.4	0–141.2	20.1	9.9	4.6	1.5	110.5	32.4	<i>p</i> = 0.0001
High doses (1.04–3.1 Gy)									
1	0.3–50.5	0–61.2	4.1	8.1	1.3	1.9	15.1	23.5	<i>p</i> = 0.0001
2	0–121.6	0–66.2	12.4	3.5	3.3	0.1	36.8	17.9	<i>p</i> = 0.0001
3	0–248	0–141.2	27.1	9.9	5.9	1.5	98.8	32.4	<i>p</i> = 0.0001
4	0–227.7	0–65.8	8.7	3.6	2.4	0.8	35.1	10.7	<i>p</i> = 0.0001
5	0–174.3	0–119.1	11.3	6.6	2.3	0.06	41.7	32.6	<i>p</i> = 0.0001

Table compiled by the authors based on their original data

Table 7. Frequency of ultra-short and ultra-long telomeres in the case-control study

Pair No.	Ultra-short telomeres, %		χ^2	<i>p</i> -value	Ultra-long telomeres, %		χ^2	<i>p</i> -value
	case	control			case	control		
Medium doses								
1	0.8	1.1	147.0	<<< 0.0001	31	2.0	460	<<< 0.0001
2	0.5	11	351	<<< 0.0001	33	9.7	294	<<< 0.0001
3	3.3	4	1.7	0.19	1.8	0.2	32.3	<<< 0.0001
4	0.47	1.9	17.8	<<< 0.0001	39	9.7	566.2	<<< 0.0001
High doses								
1	0.8	0.7	0.03	0.86	1.4	2.9	8.0	0.005
2	1	11	150	<<< 0.0001	3.2	2	135	<<< 0.0001
3	0.38	1.9	20	<<< 0.0001	14	9.7	20.7	<<< 0.0001
4	0.4	4	57.2	<<< 0.0001	9	0.2	227.7	<<< 0.0001
5	0.5	11	179.6	<<< 0.0001	13.5	9.7	11.2	0.0008

Table compiled by the authors based on their original data

Note: *p*-value — level of statistical significance of the differences; χ^2 — Pearson's chi-squared test.

Table 7 presents the frequencies of ultra-short and ultra-long telomeres for each case-control pair (based on reference values) among individuals with medium and high bone marrow doses.

The study yielded statistically significant data on changes in the frequency of ultra-short telomeres in three out of four pairs ($p < 0.0001$), with the exception of Pair No. 3 ($p = 0.19$). At the same time, in all examined pairs,

the frequency of occurrence of ultra-short telomeres in exposed donors was statistically significantly lower than that in the controls. Furthermore, the frequency of ultra-long telomeres in all pairs of donors exposed to doses of 0.14–0.86 Gy was statistically significantly higher compared to the control group ($p < 0.0001$).

It can be seen that for individuals exposed in the dose range of 1.04–3.1 Gy, the frequency of ultra-short telomeres is statistically significantly lower relative to the control in four out of five pairs. No statistically significant differences in the frequency of ultra-short telomeres were observed in Pair No. 1. Also, in this same pair, the exposed donor had a lower frequency of ultra-long telomeres compared to the control. In the remaining four examined pairs, an increase in the frequency of ultra-long telomeres was observed in exposed individuals relative to the controls.

Subsequently, for the donors selected into the case-control study, Spearman's rank correlation coefficient was used to assess the relationship between the proportion of ultra-short telomeres and the dose. The results found no correlation between ultra-long telomeres and bone marrow dose; however, a trend towards a decrease in the frequency of ultra-short telomeres with an increase in bone marrow dose was observed. A statistically significant inverse correlation of moderate strength was established between the frequency of ultra-short and ultra-long telomeres in exposed donors ($r_s = -0.654$, $p = 0.004$).

Subsequently, the most suitable regression model describing the dependence of ultra-short telomere frequency on bone marrow dose was fitted (Figure).

The Figure shows that an increase in dose is associated with an exponential decrease in the number of ultra-short telomeres.

$$y = 4.3 e^{-1.5D} [R^2 = 0.23, p = 0.0036], \quad (2)$$

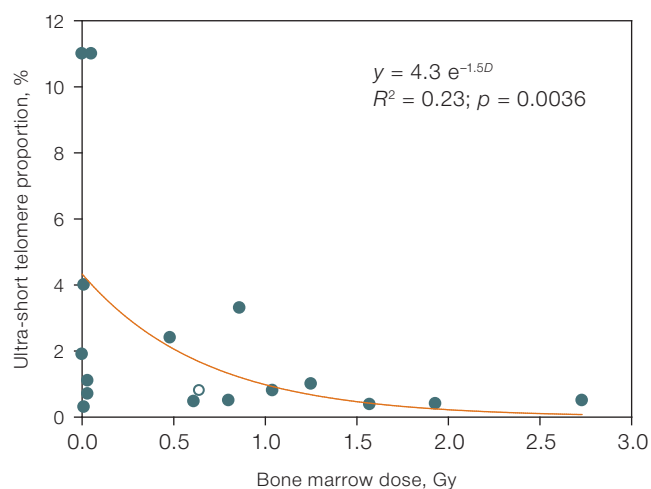


Figure prepared by the authors

Fig. Regression dependence of the percentage of ultra-short telomeres on bone marrow dose

y — frequency of ultra-short telomeres, D — bone marrow dose, Gy.

Thus, it was demonstrated that exposed individuals exhibit a reduced frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres relative to the comparison group. An exponential decrease in the frequency of ultra-short telomeres with an increase in bone marrow dose was observed.

DISCUSSION

The present study continues a project investigating the effects of chronic radiation exposure on chromosomal telomeric regions. As part of this long-term project, the length of telomeric regions in individual chromosome pairs in chronically exposed individuals was examined, and the frequency of inversions involving telomeric regions and the frequency of telomeric region loss in metaphase chromosomes from cultured peripheral blood T cells of exposed residents in the Southern Urals were assessed [14]. To that end, a method of fluorescent staining of chromosomal telomeric regions was proposed and validated. The use of the Q-FISH method is justified by its capacity to investigate telomeric regions in each chromosome within a donor's cells. This enables the characterization of the telomere profile.

The present study is the first of its kind to conduct an analysis of the influence of both radiation and non-radiation factors (age) on the frequency of ultra-short and ultra-long telomeres. The frequency of ultra-short and ultra-long telomeres in exposed individuals was assessed based on reference values for telomeric region length, which were calculated for a comparison group of a similar age range. The reference range for telomere length in the comparison group (age 71–83 years) was 0.7–25.6%. Telomeres with a length $< 0.7\%$ were classified as ultra-short, while those above 25.6% were classified as ultra-long.

A statistically significant increase in median telomere length was demonstrated in the group of exposed individuals compared to the comparison group. At the same time, wide variability in telomere length was observed in both exposed individuals and the comparison group, which is consistent with previously obtained data [1, 15]. This variability may indicate the circulation of T cells with different replicative ages in the peripheral blood.

According to the results obtained, in the overall sample of exposed individuals, the frequency of ultra-short telomeres was lower, and that of ultra-long telomeres was higher relative to the comparison group. These findings were further supported by an assessment using the case-control method with age-matched donors. The presence of longer telomeres in exposed individuals may indicate molecular-genetic features associated with either increased telomerase activity

[16] or epigenetic changes in other enzymes regulating telomere replication and repair [17, 18], as well as the selection of more radioresistant cells and the death of radiosensitive ones [4]. The donor selection criteria for the cytogenetic study may have also played a role, since the sample inherently comprised the healthiest individuals relative to the general population. This is a necessary selection criterion, since excluded medical conditions, therapeutic procedures, and medications can distort cytogenetic data [1].

Furthermore, a statistically significant decrease in median telomere length was observed in the donor group with a high bone marrow dose compared to the group exposed to medium doses. This difference was primarily associated with a reduced frequency of ultra-long telomeres in the high-dose group, while the frequency of ultra-short telomeres remained at the same level as in individuals with medium doses. At the same time, the frequency of ultra-long telomeres did not depend on the bone marrow dose, whereas the frequency of ultra-short telomeres decreased exponentially with an increase in dose. The presented patterns require further in-depth investigation. Provided that subsequent studies confirm the relationship between the frequency of ultra-short telomeres and radiation dose, this parameter could be used as an indicator of radiation exposure or a biomarker of cell radiosensitivity [19, 20].

CONCLUSION

The present study provides data on the influence of chronic radiation exposure on telomere length in women, obtained using the Q-FISH method. This method enables the determination of relative telomeric region length by calculating the ratio of the telomeric signal fluorescence intensity to the centromeric signal fluorescence intensity of chromosome 2, expressed as a percentage (T/C, %). Ultra-short and ultra-long telomeres in exposed individuals were identified relative to the reference telomere length value calculated in the comparison group.

Our results revealed a statistically significant increase in median telomere length in exposed individuals relative to the comparison group. The increase in telomere length is associated with a decreased frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres in exposed individuals. The study conducted using a case-control design with age-matched donors confirmed that this pattern persists independently of age, underscoring the significance of the radiation factor. The dependence of ultra-short telomere frequency on bone marrow dose is described by a non-linear regression manifested in an exponential decrease in the frequency of ultra-short telomeres with an increase in dose. This phenomenon highlights the importance of further investigation into the role of ultra-short telomeres as a potential marker of radiation exposure.

References

1. Akhmadullina YuR, Vozilova AV, Krivoshchapova YV. The effect of chronic exposure on the parameters of cytogenetic markers of senescence in the residents of the Techa riverside settlements. *Extreme Medicine*. 2024;26(2):56–66 (In Russ.). <https://doi.org/10.47183/mes.2024.018>
2. Baird DM, Rowson J, Wynford-Thomas D, Kipling D. Extensive allelic variation and ultrashort telomeres in senescent human cells. *Nature Genetics*. 2003;33(2):203–7. <https://doi.org/10.1038/ng1084>
3. Graakjaer J, Londono-Vallejo JA, Christensen K, Kølvrå S. The pattern of chromosome-specific variations in telomere length in humans shows signs of heritability and is maintained through life. *Annals of the New York Academy of Sciences*. 2006;1067:311–6. <https://doi.org/10.1196/annals.1354.042>
4. Berardinelli F, Nieri D, Sgura A, Tanzarella C, Antocchia A. Telomere loss, not average telomere length, confers radiosensitivity to TK6-irradiated cells. *Mutation Research*. 2012;740(1–2):13–20. <https://doi.org/10.1016/j.mrfmmm.2012.11.004>
5. Lustig A, Shterev I, Geyer S, Shi A, Hu Y, Morishita Y, et al. Long term effects of radiation exposure on telomere lengths of leukocytes and its associated biomarkers among atomic-bomb survivors. *Oncotarget*. 2016;7(26):38988. <https://doi.org/10.18632/oncotarget.8801>
6. Reste J, Zvigule G, Zvagule T, Kurjane N, Eglite M, Gabruseva N, et al. Telomere length in Chernobyl accident recovery workers in the late period after the disaster. *Journal of Radiation Research*. 2014;55(6):1–12. <https://doi.org/10.1093/jrr/rru060>
7. Scherthan H, Sotnik N, Peper M, Schrock G, Azizova T, Abend M. Telomere Length in Aged Mayak PA Nuclear Workers Chronically Exposed to Internal Alpha and External Gamma Radiation. *Radiation Research*. 2016;185(6):658–67. <https://doi.org/10.1667/RR14271.1>
8. Slijepcevic P. Is there a link between telomere maintenance and radiosensitivity? *Radiation Research*. 2004;161(1):82–6. <https://doi.org/10.1667/rr3093>
9. Fiesco-Roa MÓ, García B, Leal-Anaya P, van 't Hek R, Wegman-Ostrosky T, Frías S, et al. Fanconi anemia and dyskeratosis congenita/telomere biology disorders: Two inherited bone marrow failure syndromes with genomic instability. *Frontiers in Oncology*. 2022;12:949435. <https://doi.org/10.3389/fonc.2022.949435>
10. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell*. 2001;107(1):67–77. [https://doi.org/10.1016/s0092-8674\(01\)00504-9](https://doi.org/10.1016/s0092-8674(01)00504-9)
11. Cagsin H, Uzan A, Tosun O, Rasmussen F, Serakinci N. Tissue-Specific Ultra-Short Telomeres in Chronic Obstructive Pulmonary Disease. *International Journal of Chronic Obstructive Pulmonary Disease*. 2020;15:2751–7. <https://doi.org/10.2147/COPD.S267799>
12. Akleev AV, ed. *The consequences of radioactive contamination of the Techa River*. Chelyabinsk: Kniga; 2016 (In Russ.).
13. Shishkina EA, Napier BA, Preston DL, Degteva MO. Dose estimates and their uncertainties for use in epidemiological studies of radiation-exposed populations in the Russian Southern Urals. *PLoS One*. 2023;18(8):e0288479. <https://doi.org/10.1371/journal.pone.0288479>

14. Krivoshchapova YV. Estimation of the impact of chronic radiation exposure on telomere loss in women's T lymphocytes. *Bulletin of RSMU*. 2024;6:172–8 (In Russ.). <https://doi.org/10.24075/brsmu.2024.055>
15. Krivoshchapova YaV, Vozilova AV. The study of the telomere length of the chromosomes in T-lymphocytes of the exposed individuals. *Radiation Safety Problems*. 2022;3(107):71–96 (In Russ.). EDN: [FPBYYE](#)
16. Yasumoto S, Kunitura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, et al. Telomerase activity in normal human epithelial cells. *Oncogene*. 1996;13(2):433–9.
17. Drosopoulos WC, Deng Z, Twayana S, Kosiyatrakul ST, Vladimirova O, Lieberman PM, et al. TRF2 Mediates Replication Initiation within Human Telomeres to Prevent Telomere Dysfunction. *Cell Reports*. 2020;33(6):108379. <https://doi.org/10.1016/j.celrep.2020.108379>
18. Wang C, Zhao L, Lu S. Role of TERRA in the regulation of telomere length. *International Journal of Biological Sciences*. 2015;11(3):316–23. <https://doi.org/10.7150/ijbs.10528>
19. Mirjolet C, Boidot R, Saliques S, Ghiringhelli F, Maingon P, Créhange G. The role of telomeres in predicting individual radiosensitivity of patients with cancer in the era of personalized radiotherapy. *Cancer Treatment Reviews*. 2015;41(4):354–60. <https://doi.org/10.1016/j.ctrv.2015.02.005>
20. Stanley SE, Rao AD, Gable DL, McGrath-Morrow S, Armanios M. Radiation Sensitivity and Radiation Necrosis in the Short Telomere Syndromes. *International Journal of Radiation Oncology, Biology, Physics*. 2015;93(5):1115–7. <https://doi.org/10.1016/j.ijrobp.2015.08.048>

Authors' contributions. All authors confirm that their contributions meet the ICMJE criteria for authorship. The primary contributions are distributed as follows: Yana V. Krivoshchapova — research concept and design, conducting laboratory investigations, acquisition of primary data, statistical analysis of results, drafting the article; Yulia R. Akhmadullina — statistical analysis and interpretation of data, writing, editing, and final preparation of the manuscript.

AUTHORS

Yana V. Krivoshchapova

<https://orcid.org/0000-0002-2555-2616>
yana_ho@mail.ru

Yulia R. Akhmadullina, Cand. Sci. (Biol.)

<https://orcid.org/0000-0003-4394-2228>
akhmadullina.yul@yandex.ru

<https://doi.org/10.47183/mes.2025-345>



SIGNIFICANCE OF QT INTERVAL PROLONGATION IN YOUTH AND ADOLESCENT SPORTS (LITERATURE REVIEW)

Leonid M. Makarov[✉], Vera N. Komoliatova, Anush G. Akopyan, Dmitryi A. Besportochnyi, Alena V. Dmitrieva, Irina I. Kiseleva, Ilya V. Zyabkin

Federal Scientific and Clinical Center for Children and Adolescents of the Federal Medical and Biological Agency, Moscow, Russia

Introduction. Up to 42% of young athletes who die suddenly show no signs of known cardiac diseases at autopsy (such as cardiomyopathies, myocarditis, or congenital heart defects). However, molecular genetic analysis in young sudden death victims identifies mutations in genes responsible for Long QT Syndrome (LQTS) in 17–23% of cases. During comprehensive medical examinations (CME) of young athletes in Russian national teams within the FMBA of Russia system, up to 24% of athletes are disqualified from sports due to detected QT interval prolongation for further diagnosis, with the diagnosis of LQTS being confirmed in 0.24% of cases.

Objective. Analysis of diagnostic methods for LQTS in young athletes and criteria for their clearance for sports training.

Discussion. The pathogenesis of LQTS is based on genetically determined impairment of cardiac ion channel function, which causes myocardial electrical instability predisposing to cardiac events. Such events include Torsades de Pointes (TdP) ventricular tachycardia, syncope, cardiac arrest, and sudden cardiac death (SCD). Diagnosis of LQTS is based on the Schwartz criteria, which incorporate data from standard ECG (QTc > 450 ms), Holter monitoring (HM), stress tests, clinical presentation, and family history. A score of more than three points based on these criteria makes the diagnosis of LQTS highly probable. According to international criteria for QT interval assessment in adult athletes, the proposed upper limits of normal QTc duration are up to 470 ms for males and up to 480 ms for females. Some authors suggest that QTc values up to 500 ms may be acceptable in athletes; however, according to the Schwartz criteria, this value is sufficient to confirm the diagnosis of LQTS. Bradycardia, typical of trained athletes, is another LQTS criterion in pediatric ECG assessment. Methods for QT assessment during bradycardia in young athletes are not specified. Intense training may increase QT interval duration; conversely, temporary detraining may lead to its decrease. The Schwartz criteria based on HM results include only T-wave alternans and TdP tachycardia. QT interval assessment during HM remains a subject of debate. To date, at least 17 pathogenic genes responsible for LQTS have been identified. Detection of Class IV–V pathogenic mutations is sufficient for diagnosing LQTS, regardless of QT duration. The issues of clearance/return to sport-specific training for athletes with LQTS remain controversial, being addressed differently across countries. There are known cases of athletes with LQTS who have achieved significant success in sport competitions, as well as regular occurrences of SCD in young individuals with this condition. Current Russian and international guidelines state that competitive sports are contraindicated for patients with confirmed LQTS.

Conclusions. The assessment of the QT interval in young athletes involves numerous methodological and clinical peculiarities distinct from those in non-athletic individuals. Underestimating these peculiarities can lead to over- or under-diagnosis of LQTS, thereby potentially creating a life-threatening situation for the athlete. Individual risks in different categories of LQTS patients are composed of multiple components. Disqualification from sports does not eliminate the risk of cardiac events in LQTS; at the same time, the extent to which sports activity itself increases these risks remains unknown today. This underscores the relevance of actively studying and clarifying these unresolved issues in young athletes with QT interval prolongation and LQTS.

Keywords: youth and adolescent sports; QT interval; long QT syndrome; sudden cardiac death

For citation: Makarov L.M., Komoliatova V.N., Akopyan A.G., Besportochnyi D.A., Dmitrieva A.V., Kiseleva I.I., Zyabkin I.V. Significance of QT interval prolongation in youth and adolescent sports (literature review). *Extreme Medicine*. 2026;28(1):79–87. <https://doi.org/10.47183/mes.2025-345>

Funding: the study was carried out without sponsorship.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Leonid M. Makarov dr.leonidmakarov@mail.ru

Received: 8 July 2025 **Revised:** 17 Sep. 2025 **Accepted:** 9 Oct. 2025 **Online first:** 29 Oct. 2025

УДК 616.1

ЗНАЧЕНИЕ УДЛИНЕНИЯ ИНТЕРВАЛА QT В ДЕТСКО-ЮНОШЕСКОМ СПОРТЕ (ОБЗОР ЛИТЕРАТУРЫ)

Л.М. Макаров[✉], В.Н. Комолятова, А.Г. Акопян, Д.А. Беспорточный, А.В. Дмитриева, И.И. Киселева, И.В. Зябкин

Федеральный научно-клинический центр детей и подростков Федерального медико-биологического агентства, Москва, Россия

Введение. До 42% юных спортсменов, умерших внезапно, не имели каких-либо признаков известных болезней сердца при аутопсии (кардиомиопатий, миокардитов или пороков сердца), а молекулярно-генетический анализ, проведенный у молодых внезапно погибших лиц, выявляет в 17–23% случаев мутации в генах, отвечающих за синдром удлиненного интервала QT (СУИQT). При проведении углубленных медицинских обследований (УМО) у юных спортсменов сборных РФ в системе ФМБА России до 24% атлетов отводятся от спорта из-за выявленного удлинения интервала QT для уточнения диагноза и в 0,24% случаев диагноз СУИQT подтверждается.

Цель. Анализ методов диагностики удлинения интервала QT у юных спортсменов и критериев их допуска к спортивным тренировкам.

Обсуждение. В основе развития СУИQT лежит генетически детерминированное поражение функции ионных каналов кардиомиоцитов, вызывающее электрическую нестабильность миокарда, которая предрасполагает к возникновению сердечных событий: желудочковой тахикардии типа «пируэт» (Torsade de Pointes — TdP), синкопе, остановке сердца и внезапной сердечной смерти (ВСС).

© L.M. Makarov, V.N. Komoliatova, A.G. Akopyan, D.A. Besportochnyi, A.V. Dmitrieva, I.I. Kiseleva, I.V. Zyabkin, 2025

Для диагностики СУИQT используются критерии Шварца, в которые входят данные стандартной ЭКГ (QTc более 450 мс), холтеровского мониторирования (ХМ), стресс-тестов, клинической картины, семейного анамнеза. Выявление более 3 баллов по этим критериям делает диагноз СУИQT высоковероятным. Согласно международным критериям по оценке интервала QT у взрослых спортсменов, нормой продолжительности интервала QTc предлагают считать у мужчин значения QTc до 470 мс, а у женщин — до 480 мс. Некоторые авторы предлагают считать допустимыми значения QTc у спортсменов до 500 мс, но по критериям Шварца этого достаточно для подтверждения диагноза СУИQT. Брадикардия, типичная для тренированных спортсменов, также является одним из критериев СУИQT при оценке ЭКГ у детей. Методы оценки QT при брадикардии у юных спортсменов не уточнены. Интенсивные тренировки могут увеличивать продолжительность интервала QT, а временный детренинг, наоборот, уменьшать. В критерии Шварца по результатам ХМ включены только альтернация зубца Т и тахикардии TdP. Оценка интервала QT при ХМ является предметом дискуссий. На сегодняшний день известно не менее 17 патогенных генов, отвечающих за формирование СУИQT. Выявление патогенных мутаций IV–V класса достаточно для постановки диагноза СУИQT, независимо от продолжительности QT. Вопросы допуска/возвращения в спорт спортсменов с СУИQT остаются дискуссионными и решаются в разных странах неодинаково. Известны как спортсмены с СУИQT, добившиеся значительных успехов в спорте, так и регулярные случаи ВСС у молодых людей с этим заболеванием. Существующие на сегодняшний день отечественные и международные рекомендации говорят о противопоказании для занятий соревновательным спортом больным с доказанным СУИQT.

Выводы. В оценке интервала QT у юных спортсменов существует много методических и клинических особенностей, в отличие от лиц, не занимающихся спортом. Их недооценка может привести к гипер- или гиподиагностике СУИQT и создать угрозу для жизни спортсмена. Индивидуальные риски у разных категорий больных СУИQT слагаются из многих компонентов. Отвод от спорта не устраняет риск развития сердечных событий при СУИQT, но насколько сам спорт увеличивает риски, сегодня неизвестно. Это определяет актуальность активного изучения и уточнения данных неопределенных вопросов у юных спортсменов с удлинением интервала QT и СУИQT.

Ключевые слова: детско-юношеский спорт; интервал QT; синдром удлиненного интервала QT; внезапная сердечная смерть

Для цитирования: Макаров Л.М., Комолятова В.Н., Акопян А.Г., Беспорточный Д.А., Дмитриева А.В., Киселева И.И., Зябкин И.В. Значение удлинения интервала QT в детско-юношеском спорте (обзор литературы). *Медицина экстремальных ситуаций*. 2026;28(1):79–87. <https://doi.org/10.47183/mes.2025-345>

Финансирование: исследование проведено без спонсорской поддержки.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Макаров Леонид Михайлович dr.leonidmakarov@mail.ru

Статья поступила: 08.07.2025 **После доработки:** 17.09.2025 **Принята к публикации:** 09.10.2025 **Online first:** 29.10.2025

INTRODUCTION

Electrocardiographic (ECG) changes constituting the “athlete’s heart” phenotype are predominantly studied in adult athletes. A limited number of studies are dedicated to ECG findings in adolescent athletes engaged in competitive sports [1]. The QT interval is one of the most critical parameters in the ECG assessment of young athletes, since its pathological prolongation serves as a marker for the risk of dangerous ventricular arrhythmias (“red flag”) necessitating mandatory further investigation and clarification of its underlying causes [2, 3].

The clinical significance of QT interval prolongation in athletes is determined primarily by its association with the problem of sudden cardiac death (SCD). Up to 42% of young athletes who experience a sudden death show no signs of known cardiac diseases at autopsy (such as cardiomyopathies, myocarditis, or congenital heart defects) [4]. Molecular genetic analysis performed in young sudden death victims without obvious cardiac disease identified at autopsy or in their relatives reveals mutations in genes responsible for long QT syndrome in 17–23% of cases [5, 6].

Long QT syndrome (LQTS) is a condition with a high risk of life-threatening cardiac events. These include polymorphic Torsade de Pointes (TdP) ventricular tachycardia, syncope, cardiac arrest, and sudden cardiac death (SCD). The disease is based on a genetically

determined impairment of cardiac ion channel function (a hereditary or congenital channelopathy), which causes electrical instability of the cardiac cell and the entire myocardium, predisposing to the occurrence of cardiac events [7]. QT interval prolongation on ECG is detected in 24% of young athletes during comprehensive medical examinations (CME) at the elite sports mastery level within the FMBA of Russia system, leading to temporary or permanent disqualification from sports to clarify its nature and rule out LQTS [8]. In 0.24% of cases, the LQTS diagnosis is confirmed based on clinical electrocardiographic criteria and molecular genetic studies [9], which raises new questions regarding potential risks, clearance for sports participation, and the necessity of treatment.

This article carries out a review of methods for diagnosing QT interval prolongation in young athletes and the criteria for their clearance for sports training.

MATERIALS AND METHODS

The literature search was conducted in electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Web of Science, Scopus, PubMed) languages. The search depth was 21 years. Articles published in medical scientific journals and specialized medical monographs relevant to the research topic were analyzed. The criteria for source selection

and inclusion in the review were based on search queries using the following keywords: QT interval, sports, young elite athletes, long QT syndrome, acquired long QT syndrome, clearance for sports.

RESULTS AND DISCUSSION

LQTS was first described in 1957 by Norwegian physicians A. Jervell and F. Lange-Nielsen [10]. They observed a family with six children, four of whom had congenital sensorineural deafness and episodes of loss of consciousness; subsequently, three of them died suddenly. The current prevalence of LQTS is estimated to be one in 2000–2500 individuals, making it one of the most common arrhythmogenic channelopathies [11].

The Schwartz criteria [12, 13] are used worldwide for diagnosing LQTS (Table 1). These criteria incorporate data from standard ECG, Holter monitoring (HM), stress tests, clinical presentation, family history, and examination of first-degree relatives. A score of more than three points on these criteria, in the absence of secondary causes for QT prolongation, makes the diagnosis of LQTS highly probable. The identification of a pathogenic gene with pathogenicity Class IV–V is also sufficient for establishing the diagnosis of LQTS, regardless of the QT interval duration [13].

According to the clinical interpretation based on [12, 13], a total score of ≤ 1 points indicates a low probability of LQTS; a total score of 1–3 points indicates an intermediate probability of LQTS; a total score of ≥ 3 points indicates a high probability of LQTS.

The primary criterion for diagnosing the condition remains the detection of a prolonged QT interval on a standard 12-lead resting ECG. Methodologically, the QT interval measurement is recommended to be performed in standard lead II or precordial lead V5, where it is considered most representative [14]. Since the QT interval duration is dependent on heart rate (HR), several formulas exist for heart rate correction of the QT interval.

Clinical medicine, including sports cardiology, primarily uses the Bazett formula [15]:

$$QTc \text{ (ms)} = QT \text{ (ms)} / \sqrt{RR \text{ (s)}}, \quad (1)$$

where QTc — corrected QT interval value in milliseconds (ms); QT — measured QT interval value in milliseconds (ms); RR — RR interval value in seconds (s), preceding the measured QT interval.

For children, a normal QTc interval value on a standard ECG is considered to be less than 440 ms [16]. For adult males, a QTc value of less than 450 ms is proposed as the norm, while for females, it is less than 460 ms [17]. Several studies suggest the preferential use of the Fridericia formula for QTc assessment in athletes due to employing a cubic root rather than a square root [18]. In the study [19] involving a large cohort of

1473 adolescents aged 7–15 years engaged in sports, the QTc interval calculated using the Bazett formula was 412 ± 25 ms, whereas the QT interval corrected using the Fridericia formula was 387 ± 21 ms. The authors concluded that the Fridericia formula is a more accurate method for assessing the QT interval duration in young athletes. However, despite the criticism of QTc assessment using the Bazett formula, precise reference values for QTc calculated using alternative formulas have not been established for young athletes, thus requiring further research [20].

According to current international criteria for assessing the QT interval in adult elite athletes, the proposed upper limits of the Bazett-corrected normal QTc interval are 470 ms for males and 480 ms for females [15]. Some studies in athletes suggest that QTc values up to 500 ms may be considered acceptable and non-hazardous [21, 22]. However, according to the Schwartz criteria (Table 1), the detection of a QTc interval in the range of 460–480 ms already contributes three points to the LQTS diagnostic score and is assessed as indicating an

Table 1. Schwartz criteria for diagnosis of long QT syndrome

Electrocardiographic Criteria	Score
QTc > 480 ms	3
QTc > 460–479 ms	2
QTc > 450–459 ms (males)	1
QTc $\geq$ 480 ms at the 4th min of recovery during stress testing (cycle ergometry, treadmill)	1
Torsade de Pointes tachycardia	1
Macroscopic T-Wave Alternans	1
Bifid T Wave in at least three leads	1
Bradycardia (age-appropriate)	0.5
Clinical criteria	
Stress-induced syncope	2
Syncope without stress	1
Congenital deafness	0.5
Documented family history of LQTS	1
Family history of unexplained sudden death in relatives under 30 years of age	0.5

Table compiled by the authors based on data from the sources [12, 13]

Note: QTc — corrected QT interval value in milliseconds (ms), calculated by the formula $QTc \text{ (ms)} = QT \text{ (ms)} / \sqrt{RR \text{ (s)}}$; QT — measured QT interval value in milliseconds (ms); RR — RR interval value in seconds (s), preceding the measured QT interval.

intermediate probability of LQTS (Table 1). This discrepancy in interval assessment between clinical and sports cardiologists creates difficulties in reaching a diagnostic conclusion. In addition, as studies have shown, fewer than 50% of cardiologists and fewer than 40% of other physicians who interpret ECGs are capable of correctly measuring and assessing the QT interval [23].

Expert opinions on the impact of athletic training on the QT interval duration are equivocal. In a study by Małek et al. following ECG screening of 600 healthy Caucasian children (aged 5–17 years) [24], only the development of bradycardia, sinus arrhythmia, incomplete right bundle branch block, and early repolarization pattern were considered. However, Caramoci [25] reviewed 20 published studies and found that several of them confirmed a longer QT interval in young athletes compared to non-athletic individuals at the same heart rate. In another large-scale study of automated ECG data from 672 young athletes aged 17–22 years compared to 6534 non-athletes, a lower heart rate and a longer QT interval were observed. However, these athletes had a significantly shorter QTc interval = 409 (384–426) ms, calculated using the Bazett formula, versus 428 (411–445) ms in non-athletes [26]. This result may be attributable to the lower QTc values produced by this specific formula in the context of bradycardia [27]. Clinical observations have shown that intense training may increase the QT interval, whereas temporary detraining may shorten its duration [25, 28]. All researchers emphasize the need for heightened vigilance in cases of QT prolongation in athletes and the necessity of ruling out LQTS [29].

The Schwartz diagnostic criteria for LQTS include QTc assessment at the 4th minute of recovery after a stress test (Table 1). Other criteria for QT evaluation have been proposed in [30–32]; however, these criteria have not been validated in athlete cohorts. Holter monitoring (HM) is also used in the diagnosis of LQTS according to the Schwartz criteria. However, based on HM results, the Schwartz criteria include only the documentation of Torsades de Pointes tachycardia and macroscopic T-wave alternans. The measurement and interpretation of the QT interval itself during HM remain a subject of debate. Some authors recommend measuring the QT interval at a stable heart rate of 60 bpm without correction [31], while others propose QT assessment at minimum and maximum heart rates [32, 33]. In addition, some approaches suggest using an average daily QTc greater than 450–460 ms as a diagnostic criterion [33–35]. Such studies have not thus far been conducted in athletes.

Evaluation of QT dynamics based on calculating the linear regression coefficient between RR and QT intervals (Slope QT/RR) is an additional method for QT interval assessment during HM in athletes [36]. QT dynamics reflects the adaptation of the QT interval to changes in heart rate. In healthy individuals, the normal linear regression coefficient (Slope QT/RR) ranges 0.16 ± 0.02 for men and 0.20 ± 0.04 for women [37]. Trained healthy

athletes are characterized by lower Slope QT/RR values: 0.13 ± 0.02 for men and 0.16 ± 0.03 for women [37]. In physiological terms, lower Slope QT/RR values are defined as QT hypo-adaptation. This implies that under an increase in heart rate, the corrected QT interval (QTc) prolongs beyond the normal range; under a decrease in heart rate, it fails to shorten to normal values [36]. Conversely, QT hyper-adaptation (high Slope QT/RR values) leads to significant QT interval shortening at higher heart rate values and excessive prolongation under lower heart rate values [38]. QT hyper-adaptation is typical under conditions of increased sympathetic influence on heart rhythm in newborns [39], in patients with type 3 long QT syndrome (LQT3), and in chronic heart failure [39], where cardiac events occur at rest, during sleep, or immediately after physical exertion in athletes [27]. This suggests possible gene-specific individual risks associated with sports participation in patients with LQTS.

Bradycardia, typical of trained athletes, is one of the LQTS criteria included in the Schwartz score with a specific diagnostic weight of 0.5 points. This item is considered only in children and adolescents, where heart rate norms have stricter age-specific limits [16]. On the other hand, bradycardia is common in elite young athletes; as a result, when calculating QTc using the Bazett formula, it can yield a false-negative result, showing normal QTc values despite actual QT prolongation [27]. The most recent international recommendations for ECG interpretation in athletes [15] suggest performing an ECG after mild exercise in cases of bradycardia below 60 bpm, although not mentioning specific types of exercise. Viskin [40] proposed recording an ECG in the orthostatic position to increase heart rate and assess QTc during bradycardia. This study was conducted in adult non-athlete patients, and its methodology is often criticized [30].

To date, at least 17 pathogenic genes responsible for LQTS have been identified [11–13] (Table 2).

The three most frequently occurring molecular genetic variants of LQTS are: Type 1 (LQT1) and Type 2 (LQT2), which are associated with mutations in the *KCNQ1* and *KCNH2* genes, respectively, governing potassium channel function. Type 3 (LQT3) is associated with mutations in the *SCN5A* gene, which regulates sodium channel function in cardiomyocytes (Table 2). These three variants account for approximately 80% of all LQTS patients [11–13].

The genetic variant of LQTS can often be suspected based on a characteristic ECG pattern. For patients with LQT1, the ECG typically shows a broad T-wave. In this variant, the primary triggers for cardiac events are physical exertion, water contact, and swimming. For individuals with LQT2, the ECG is characterized by a bifid T-wave, and a typical trigger for cardiac events in this case is a sudden loud sound. In women, the first episodes often occur after childbirth. Finally, for the LQT3 variant, the ECG typically shows a late-onset T-wave

Table 2. Genetic variants of long QT syndrome

Clinical syndrome	Molecular genetic variant of LQTS ^e	Inheritance pattern	Locus	Ion channel	Defective gene	Lethal arrhythmia
LQTS (RW)	LQT1	AD	11p15	'KS	<i>KCNQ1, KvLQT1</i>	TdP
	LQT2		7q35	I Kr	<i>KCNH2, HERG</i>	
	LQT3		3p21	'Na	<i>SCN5A, Nav1.5</i>	
	LQT4		4q25		<i>ANKB, ANK2</i>	
	LQT5		21q22	'KS	<i>KCNE1, minK</i>	
	LQT6		21q22	I Kr	<i>KCNE2, MiRP 1</i>	
Anderson-Tawil Syndrome	LQT7		17q23	IK1	<i>KCNJ2, Kir 2.1</i>	
Timothy Syndrome	LQT8		6q8A	'ca-L	<i>CACNA1C, Cav1.2</i>	
	LQT9		3p25	'Na	<i>CAV3, Caveolin-3</i>	
	LQT10		11q23.3	'Na	<i>SCN4B, Navb4</i>	
	LQT11		7q21-q22	K	<i>ARAP9</i>	
	LQT12		3p25	Na	<i>Cav3a</i>	
LQTS (JLN)		AR	11p15	'KS	<i>KCNQ1, KvLQT1</i>	
			21q22	'KS	<i>KCNE1, minK</i>	

Table compiled by the authors based on data from the sources [11–13]

Note: LQTS — long QT syndrome; RW — Romano-Ward syndrome; JLN — Jervell and Lange-Nielsen syndrome; AD — autosomal dominant inheritance; AR — autosomal recessive inheritance; TdP — Torsades de Pointes polymorphic ventricular tachycardia.

with a prolonged ST segment [12]. Cardiac events in LQT3 occur more frequently during sleep, at rest, or immediately after cessation of physical exercise [11–13].

In 25–50% of individuals with an identified pathogenic mutation causing LQTS, a normal QT interval duration is observed on the baseline ECG [11–13]. In addition to congenital forms of LQTS, the QT interval duration can be prolonged by the use of certain medications, various metabolic disturbances, and the adoption of various diets (which is often the case in sports) [41, 42]. Secondary QT prolongation in athletes can be no less dangerous than congenital forms and cause life-threatening cardiac events as well.

The first step in the management of patients with QT interval prolongation is the exclusion of secondary causes (discontinuation of QT-prolonging medications, correction of potential electrolyte imbalances, etc.). In patients with confirmed LQTS, the current guidelines recommend avoiding genotype-specific potential triggers for cardiac events (e.g., physical exertion and sports, supervising children around water for Type 1, avoiding sudden loud noises for Type 2, etc.) [11–13].

The cornerstone of pharmacological therapy for all LQTS patients (even asymptomatic ones) is

beta-blocker administration. The most effective agents in preventing arrhythmic risk are non-selective beta-blockers lacking intrinsic sympathomimetic activity, such as nadolol and propranolol [11–13]. In Russian clinical practice, atenolol is widely used [43, 44]. When prescribing these medications to athletes, it is crucial to remember that they are prohibited in certain sports.¹ For patients with the Type 3 molecular genetic variant of LQTS (mutation in the *SCN5A* gene), sodium channel blockers (such as mexiletine, flecainide, and allapinin) can be used [11–13, 43].

The second stage of treatment (or in cases where beta-blockers are contraindicated) for patients with symptomatic LQTS involves considering the possibility of implantable cardioverter-defibrillator (ICD) placement [11–13]. ICD implantation is recommended as a method for secondary prevention of SCD, in addition to beta-blocker therapy, for patients who have experienced cardiac arrest [11–13]. The issue of eligibility for competitive sports participation in patients with implanted antiarrhythmic devices has not yet been definitively resolved [45]. Left cardiac sympathetic denervation (LCSD) is another therapeutic option under consideration [46]. This procedure is performed to eliminate asymmetric

¹ Independent National Anti-Doping Organization RUSADA. <https://rusada.ru/substances/prohibited-list/>

sympathetic innervation of the heart, which is a proven arrhythmogenic factor in these patients.

The issues of clearance/return to sports activities for athletes with LQTS remain controversial and are addressed differently across countries. Leading UK sports cardiologists Basavarajaiah and Sharma [47] published a case report of a 16-year-old female long-distance runner, a member of the national team. Her training volume was 18 h per week. During pre-participation screening (which had apparently not been conducted previously), her resting ECG revealed sinus bradycardia of 38 bpm, a QT interval prolonged to 620 ms, and a QTc of 530 ms. Molecular genetic analysis identified a mutation typical of Type 1 LQTS (LQT1). Her 13-year-old brother, who was actively engaged in football, was also evaluated as part of family screening; his ECG showed a QTc prolonged to 520 ms and the same genetic mutation. Both athletes were disqualified from sports participation with a recommendation to initiate beta-blocker therapy.

In Type 1 LQTS, the primary trigger for fatal arrhythmias is physical exertion, and one of the key criteria in risk stratification is a QTc prolongation >500 ms. Both factors were present in the siblings, yet no cardiac events had occurred prior to their evaluation. These and similar observations have likely shaped the following perspective on clearance for competitive sports in athletes with LQTS, being supported by leading global sports cardiologists [48]. For individuals with a positive phenotype and a QTc interval prolonged beyond 490 ms, competitive sports are prohibited; only routine daily activities (leisure-time activity) are permitted, with avoidance of genotype-specific cardiac event triggers (e.g., swimming, sudden loud noises). For asymptomatic carriers of LQT1 mutations, competitive sports may be possible provided swimming/diving is avoided. For carriers of LQTS mutations who lack the disease phenotype (i.e., no ECG changes or clinical manifestations), competitive sports are permissible. The required scope of specific evaluation includes a thorough history, ECG, exercise stress testing, 24-h HM, genetic testing, and regular annual follow-up [48]. However, reaching a final decision regarding sports participation in LQTS often remains challenging in practice.

Thus, during an international consultation regarding the management of a 16-year-old asymptomatic professional athlete (hockey) with confirmed Type 3 Long QT Syndrome (LQT3) and a QTc interval prolongation greater than 500 ms, the opinions of the aforementioned experts on continuing the athletic career were divided [49]. S. Sharma, Head of the Sports Cardiology Clinic at St. George's University (London, UK), expressed an opinion in favor of clearing the athlete for competitive sports. His argument was based on the fact that the QT interval in LQT3 shortens during physical exertion. However, A. Pelliccia, Head of the Sports Cardiology Clinic at the Institute of Sports Medicine (Rome, Italy), provided a

conclusion stating that the athlete was at high risk for cardiac events, with a verdict contraindicating participation in competitive sports [49]. A. Moss, Founder and Director of the International Long QT Syndrome Registry (Rochester, USA), also expressed the opinion that this athlete was at high risk for cardiac events and required an implantable cardioverter-defibrillator (ICD). He stated that the decision regarding the young athlete's participation in competitions should be made individually with his parents after they have been fully informed of all potential risks [49].

On the other hand, there are known athletes with LQTS who have achieved significant success in sports. For instance, Dana Vollmer, a member of the U.S. swimming team with a confirmed diagnosis of LQTS, won two gold medals at the 2012 London Olympic Games (and has a total of 32 medals from various major international competitions).² The details of her medical history and the specific variant of the syndrome are unknown; however, this case underscores the importance of a highly individualized approach to clearance of elite athletes for sports activity.

Johnson and Ackerman from the Mayo Clinic (USA) published the results of a follow-up study of a cohort comprising 130 young patients with LQTS who participated in competitive sports at various levels [50]. The patients received standard LQTS therapy with beta-blockers; some underwent implantable cardioverter-defibrillator (ICD) placement and/or left cardiac sympathetic denervation (LCSD). Not a single patient died during the seven-year follow-up period.

In the study by Aziz [51], 103 patients with confirmed LQTS who were engaged in sports (predominantly recreational) were observed. No episodes of syncope or SCD were recorded during the follow-up period. Two patients experienced ICD discharges, but these were not associated with athletic activity. On the other hand, despite some optimism from these observations, the number of athletes with this condition who have died suddenly also remains significant [52, 53]. Consequently, the current Russian official guidelines state that competitive sports are unequivocally contraindicated for patients with confirmed LQTS [54]. However, the actual risks for different categories of LQTS patients are highly individual and depend on numerous components, including clinical presentation, sex, ECG markers, specific genetic mutation characteristics, and other, still poorly understood, factors [55].

CONCLUSION

We conducted a review of studies focusing on QT interval assessment methods in young athletes, peculiarities of LQTS diagnosis, and issues of clearance for sports training and established that evaluation of the QT interval in athletes has a number of methodological

² Dalesio J. Olympian Dana Vollmer swims through heart risk. *Huffpost*. 2012. https://www.huffpost.com/entry/dana-vollmer-heart-condition_n_1711515

and clinical specific features, distinct from ECG interpretation in individuals not engaged in regular competitive sports. Underestimation of these features can lead to both overdiagnosis and unjustified disqualification from sports, as well as underdiagnosis of LQTS, which may result in a failure to identify a dangerous condition for the athlete's life.

These issues become particularly critical when making decisions about disqualifying young athletes at the elite performance level. Achieving this

level has consumed virtually the athlete's entire prior life; sports represent their life's choice and profession. Disqualification from sports does not eliminate the risk of cardiac events in LQTS; at the same time, the extent to which sports activity itself increases these risks remains unknown. This uncertainty underscores the urgency of studying and clarifying the unresolved questions regarding the evaluation, management, and permissible level of athletic activity in young athletes with QT interval prolongation and LQTS.

References

- Ragazzoni GL, Cavigli L, Cavarretta E, Maffei S, Mandoli GE, Pastore MC, et al. How to evaluate resting ECG and imaging in children practising sport: a critical review and proposal of an algorithm for ECG interpretation. *European Journal of Preventive Cardiology*. 2023;30(5):375–83. <https://doi.org/10.1093/eurjpc/zwac218>
- Corrado D, Pelliccia A, Bjørnstad HH, Vanhees L, Biffi A, Borjesson M, et al. Cardiovascular pre-participation screening of young competitive athletes for prevention of sudden death: proposal for a common European protocol. Consensus Statement of the Study Group of Sport Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. *European Heart Journal*. 2005;26(5):516–24. <https://doi.org/10.1093/eurheartj/ehi108>
- Zeppilli P, Biffi A, Cammarano M, Corrado D, Cavarretta E, Cecchi F, et al. Italian Cardiological Guidelines (COCIS) for Competitive Sport Eligibility in athletes with heart disease: update 2024. *Minerva Medica*. 2024;115(5):533–64. <https://doi.org/10.23736/s0026-4806.24.09519-3>
- Finocchiaro G, Papadakis M, Robertus JL, Dhutia H, Steriotis AK, Tome M, et al. Etiology of Sudden Death in Sports: Insights from a United Kingdom Regional Registry. *JACC*. 2016;67:2108–15. <https://doi.org/10.1016/j.jacc.2016.02.062>
- Wong LC, Roses-Noguer F, Till JA, Behr ER. Cardiac evaluation of pediatric relatives in sudden arrhythmic death syndrome: a 2-center experience. *Circulation. Arrhythmia and Electrophysiology*. 2014;7(5):800–6. <https://doi.org/10.1161/CIRCEP.114.001818>
- Behr ER, Dalageorgou C, Christiansen M, Syrris P, Hughes S, Tome Esteban MT, et al. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. *European Heart Journal*. 2008;29(13):1670–80. <https://doi.org/10.1093/eurheartj/ehn219>
- Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. *Circulation. Arrhythmia and Electrophysiology*. 2012;5(4):868–77. <https://doi.org/10.1161/circep.111.962019>
- Makarov LM, Komoliatova VN, Aksenova NV. Analysis of the reasons for exemptions from sports among young elite athletes. *Russian Bulletin of Perinatology and Pediatrics*. 2020;65(6):65–71 (In Russ.). <https://doi.org/10.21508/1027-4065-2020-65-6-65-71>
- Makarov LM, Komoliatova VN, Kiseleva II, Aksenova NV, Besportochny DA, Balykova LA, et al. Epidemiology of syncope in children and adolescents in elite sports (study EPISODE-S). *Russian Bulletin of Perinatology and Pediatrics*. 2019;64(6):62–7 (In Russ.). <https://doi.org/10.21508/1027-4065-2019-64-6-62-67>
- Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of Q-T interval and sudden death. *American Heart Journal*. 1957;54(1):59–68. [https://doi.org/10.1016/0002-8703\(57\)90079-0](https://doi.org/10.1016/0002-8703(57)90079-0)
- Schwartz P, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Tome Esteban MT, et al. Prevalence of the congenital long-QT syndrome. *Circulation*. 2009;120(18):1761–7. <https://doi.org/10.1161/circulationaha.109.863209>
- Wilde AAM, Amin AS, Postema PG. Diagnosis, management and therapeutic strategies for congenital long QT syndrome. *Heart*. 2022;108(5):332–8. <https://doi.org/10.1136/heartjnl-2020-318259>
- Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*. 2013;10:1932–63. <https://doi.org/10.1016/j.hrthm.2013.05.014>
- Lepeschkin E, Surawicz B. The measurement of the QT interval of the electrocardiogram. *Circulation*. 1952;6:378–88. <https://doi.org/10.1161/01.CIR.6.3.378>
- Sharma S, Drezner JA, Baggish A, Papadakis M, Wilson MG, Prutkin JM, et al. International recommendations for electrocardiographic interpretation in athletes. *European Heart Journal*. 2017;39:1466–80. <https://doi.org/10.1093/eurheartj/ehw631>
- Makarov LM, Kiseleva II, Komoliatova VN, Fedina NN. New norms and interpretations of children's electrocardiogram. *Pediatrics named after G.N. Speransky*. 2015;94(2):63–8 (In Russ.). EDN: TUFXDJ
- Viskin S. The QT interval: too long, too short or just right. *Heart Rhythm*. 2009;6:711–5. <https://doi.org/10.1016/j.hrthm.2009.02.044>
- Orchard JJ, Orchard JW, Raju H, La Gerche A, Puranik R, Davis A, et al. Analysis of athlete QT intervals by age: Fridericia and Hodges heart rate corrections outperform Bazett for athlete ECG screening. *Journal of Electrocardiology*. 2022;74:59–64. <https://doi.org/10.1016/j.jhl.2022.06.145>
- D'Ascenzi F, Anselmi F, Graziano F, Berti B, Franchini A, Bacci E, et al. Normal and abnormal QT interval duration and its changes in preadolescents and adolescents practicing sport. *Europace*. 2019;21(10):1566–74. <https://doi.org/10.1093/europace/euz198>
- Gupta I, Mahendran S, Davis J, Davis A, Orchard J. A Comparison of QT Interval Correction Methods on Electrocardiograms (ECG) of Athletes and Young People: A Systematic Review. *Heart, Lung and Circulation*.

- 2022;31(3):S106–7.
<https://doi.org/10.1016/j.hlc.2022.06.142>
21. Hoyte C, Terhune E, Carry P, McCanta A, Coel R. Borderline and Long QT Syndrome in Adolescent Athletes Taking Medications. *Journal of Adolescent Health*. 2015;57(2):218–21.
<https://doi.org/10.1016/j.jadohealth.2015.04.003>
 22. Basavarajaiah S, Wilson M, Whyte G, Shah A, Behr E, Sharma S. Prevalence and significance of an isolated long QT interval in elite athletes. *European Heart Journal*. 2007;28:2944–9.
<https://doi.org/10.1093/eurheartj/ehm404>
 23. Viskin S, Rosovski U, Sands AJ, Chen E, Kistler PM, Kalman JM, et al. Inaccurate electrocardiographic interpretation of long QT: the majority of physicians cannot recognize a long QT when they see one. *Heart Rhythm*. 2005;2(6):569–74.
<https://doi.org/10.1016/j.hrthm.2005.02.011>
 24. Małek ŁA, Dotka M, Brzezińska K, Pyndryk P, Materkowski M, Wiecha S. Electrocardiographic Changes in Juvenile Athletes in Relation to Sex, Age, and Sport Category. *Pediatric Cardiology*. 2025.
<https://doi.org/10.1007/s00246-025-03945-y>
 25. Caramoci A, Smaranda AM, Drăgoiu TS, Bădăraș IA. ECG Screening in Athletes: A Systematic Review of Sport, Age, and Gender Variations. *Reviews in Cardiovascular Medicine*. 2025;26(5):38209.
<https://doi.org/10.31083/RCM38209>
 26. Park J, Kimata C, Young J, Perry JC, Bratincsak A. Fine Tuning ECG Interpretation for Young Athletes: ECG Screening Using Z-score-based Analysis. *Sports Medicine — Open*. 2024;10:114.
<https://doi.org/10.1186/s40798-024-00775-9>
 27. Schnell F, Behar N, Carré F. Long-QT Syndrome and Competitive Sports. *Arrhythmia and Electrophysiology Reviews*. 2018;7(3):187–92.
<https://doi.org/10.15420/aer.2018.39.3>
 28. Dagradi F, Spazzolini C, Castelletti S, Pedrazzini M, Kotta MC, Crotti L, et al. Exercise Training-Induced Repolarization Abnormalities Masquerading as Congenital Long QT Syndrome. *Circulation*. 2020;142(25):2405–15.
<https://doi.org/10.1161/CIRCULATIONAHA.120.048916>
 29. Yanık H, Değirmenci E, Büyükkılıç B. A comprehensive electrocardiographic analysis for young athletes. *Medical and Biological Engineering and Computing*. 2021;59(9):1865–76.
<https://doi.org/10.1007/s11517-021-02401-2>
 30. Zareba W. Challenges of diagnosing long QT syndrome in patients with nondiagnostic resting QTc. *JACC*. 2010;55(18):1962–4.
<https://doi.org/10.1016/j.jacc.2010.02.018>
 31. Garson A. How to measure the QT interval—what is normal? *American Journal of Cardiology*. 1993;72(6):14B–16B.
[https://doi.org/10.1016/0002-9149\(93\)90034-a](https://doi.org/10.1016/0002-9149(93)90034-a)
 32. Eggeling T, Osterhues HH, Kochs M, Beyer M, Höher M, Hombach V. Diagnostische Wertigkeit von Standard-EKG-Methoden, Kälte-Druck-Test und Valsalva-Manöver beim idiopathischen QT-Syndrom. *Zeitschrift für Kardiologie*. 1993;82(1):1–7.
 33. Makarov L, Komoliatova V, Zaklyazminskaya E, Dmitrieva A. Ambulatory ECG Monitoring in Patients with Long QT Syndrome. *Conference: Scientific Session AHA*. Chicago; 2018.
<https://doi.org/10.13140/RG.2.2.32275.69921>
 34. Page A, Aktas MK, Soyata T, Zareba W, Couderc JP. “QT clock” to improve detection of QT prolongation in long QT syndrome patients. *Heart Rhythm*. 2016;13(1):190–8.
<https://doi.org/10.1016/j.hrthm.2015.08.037>
 35. Follansbee CW, Beerman L, Arora G. Automated QT analysis on Holter monitors in pediatric patients can differentiate long QT syndrome from controls. *PACE*. 2018;41(1):50–6.
<https://doi.org/10.1111/pace.13244>
 36. Makarov LM, Komoliatova VN, Miroshnikova EN, Kazantseva MA. Physiological significance and normative parameters of rate adaptation of QT-interval during Holter monitoring in healthy persons of young age. *Kardiologiya*. 2008;48(4):54–8 (In Russ.).
 37. Genovesi S, Zaccaria D, Rossi E, Valsecchi MG, Stella A, Stamba-Badiale M. Effects of exercise training on heart rate and QT interval in healthy young individuals: are there gender differences? *Europace*. 2007;9(1):55–60.
<https://doi.org/10.1093/europace/eul145>
 38. Makarov L, Komoliatova V, Zevald S, Schmidt G, Muller CA, Serebruany V. QT dynamicity, microvolt T-wave alternans, and heart rate variability during 24-hour ambulatory electrocardiogram monitoring in the healthy newborn of first to fourth day of life. *Journal of Electrocardiology*. 2010;43(1):8–14.
<https://doi.org/10.1016/j.jelectrocard.2009.11.001>
 39. Makarov L. The component of pathogenesis of sudden nocturnal death in patients with heart failure. *Extreme Medicine*. 2022;24(3):74–6.
<https://doi.org/10.47183/mes.2022.026>
 40. Viskin S, Postema PG, Bhuiyan ZA, Rosso R, Kalman JM, Vohra JK, et al. The response of the QT interval to the brief tachycardia provoked by standing: a bedside test for diagnosing long QT syndrome. *JACC*. 2010;55(18):1955–61.
<https://doi.org/10.1016/j.jacc.2009.12.015>
 41. Hoyte C, Terhune E, Carry P, McCanta A, Coel R. Borderline and Long QT Syndrome in Adolescent Athletes Taking Medications. *Journal of Adolescent Health*. 2015;57(2):218–21.
<https://doi.org/10.1016/j.jadohealth.2015.04.003>
 42. Yunisova AS, Smolensky AV. Acquired long QT interval in athletes. *Sports Medicine: Research and Practice*. 2021;11(4):17–25 (In Russ.).
<https://doi.org/10.47529/2223-2524.2021.4.3>
 43. Makarov LM, Komoliatova VN, Kiseleva II, Besportochnyi DA, Dmitrieva AV, Akopyan AG, et al. Long QT syndrome is a disease with a high risk of sudden death. Moscow: PH “Medpraktika-M”; 2018 (In Russ.).
 44. Makarov LM, Komoliatova VN, Zaklyazminskaya EV, Besportochnyi DA, Akopyan AG, Islanov IO, et al. 2 Cases of New Electrocardiographic Patterns in Patients with Long QT Syndrome. *JACC: Case Reports*. 2025;30(15):103753.
<https://doi.org/10.1016/j.jaccas.2025.103753>
 45. Pelliccia A. Long QT syndrome, implantable cardioverter defibrillator (ICD) and competitive sport participation: when science overcomes ethics. *British Journal of Sports Medicine*. 2014;48(15):1135–6.
<https://doi.org/10.1136/bjsports-2013-092441>
 46. Schwartz PJ, Priori SG, Cerrone M, Spazzolini C, Odero A, Napolitano C, et al. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long-QT syndrome. *Circulation*. 2004;109(15):1826–33.
<https://doi.org/10.1161/01.CIR.0000125523.14403.1E>
 47. Basavarajaiah S, Sharma S. (2009). A 16-Year-Old Female Runner with Prolonged QT Interval. *Sports Cardiology Casebook*. London: Springer; 2009.
https://doi.org/10.1007/978-1-84882-042-5_13
 48. Pelliccia A, Heidbuchel H, Corrado D, Sharma S, Björjesson M. Criteria and considerations relative to safe participation in sport for athletes with cardiac abnormalities. *The ESC Textbook of Sport Cardiology*. Oxford: Oxford university press; 2019.
<https://doi.org/10.1093/med/9780198779742.003.0041>
 49. Makarov LM. *Holter monitoring*. 4-th edition. Moscow: “Medpraktika-M”; 2017 (In Russ.).
 50. Johnson JN, Ackerman MJ. Return to play? Athletes with

- congenital long QT syndrome. *British Journal of Sports Medicine*. 2013;47(1):28–33.
<https://doi.org/10.1136/bjsports-2012-091751>
51. Aziz PF, Sweeten T, Vogel RL, Bonney WJ, Henderson J, Patel AR, et al. Sports Participation in Genotype Positive Children with Long QT Syndrome. *JACC Clinical Electrophysiology*. 2015;1(1–2):62–70.
<https://doi.org/10.1016/j.jacep.2015.03.006>
 52. Longo UG, Risi Ambrogioni L, Ciuffreda M, Maffulli N, Denaro V. Sudden cardiac death in young athletes with long QT syndrome: the role of genetic testing and cardiovascular screening. *British Medical Bulletin*. 2018;127(1):43–53.
<https://doi.org/10.1093/bmb/ldy017>
 53. Corrado D, Trovato D, Zorzi A. Long QT Syndrome: A Preventable Cause of Exercise-Induced Sudden Cardiac Death. *JACC Case Reports*. 2023;22:101991.
<https://doi.org/10.1016/j.jaccas.2023.101991>
 54. Boytsov SA, Kolos IP, Lidov PI, Smolenskiy AV, Ardashev AV, Balykova LA, et al. National recommendations for admission of athletes with cardiovascular disorders to the training and competition process. *Rational Pharmacotherapy in Cardiology*. 2011;7(6S) (In Russ.).
EDN: [OPRUET](#)
 55. Mazzanti A, Trancuccio A, Kukavica D, Pagan E, Wang M, Mohsin M, et al. Independent validation and clinical implications of the risk prediction model for long QT syndrome (1-2-3-LQTS-Risk). *Europace*. 2022;24(4):614–9.
<https://doi.org/10.1093/europace/euab238>

Authors' contributions. All authors confirm that their authorship complies with the ICMJE criteria. The primary contributions are distributed as follows: Leonid M. Makarov — conceptualization, review structuring, literature search and analysis, drafting of the initial manuscript, approval of the final manuscript; Vera N. Komoliatova — literature search and analysis regarding the impact of athletic training on the heart in children and adolescents; Anush G. Akopyan — literature search and analysis on the topic of ECG characteristics in young athletes; Dmitry A. Besportochnyi — literature search and analysis on the topic of exercise testing for QT interval assessment in athletes; Alena V. Dmitrieva — literature search and analysis on the assessment of autonomic influences on QT interval formation in athletes; Irina I. Kiseleva — literature search and analysis on the topic of sports clearance with QT interval prolongation; Ilya V. Zyabkin — final discussion, general editing.

AUTHORS

Leonid M. Makarov, Dr. Sci. (Med.)

<https://orcid.org/0000-0002-0111-3643>
dr.leonidmakarov@mail.ru

Vera N. Komoliatova, Dr. Sci. (Med.)

<https://orcid.org/0000-0002-3691-7449>
verakom@list.ru

Anush G. Akopyan

<https://orcid.org/0000-0002-4867-0594>
anushik.a.g@mail.ru

Dmitry A. Besportochnyi

<https://orcid.org/0000-0002-3699-2289>
dr.blad@mail.ru

Alena V. Dmitrieva

<https://orcid.org/0000-0002-0314-5235>
gerasimchuk_alena@mail.ru

Irina I. Kiseleva, Cand. Sci. (Med.)

<https://orcid.org/0000-0003-3285-3211>
vkis2@yandex.ru

Ilya V. Zyabkin, Dr. Sci. (Med.)

<https://orcid.org/0000-0002-9717-5872>
adm@kidsfmba.ru

<https://doi.org/10.47183/mes.2025-367>

EFFECT OF RHYTHMIC PERIPHERAL MAGNETIC STIMULATION ON THE FUNCTIONAL CAPABILITIES OF THE MUSCULOSKELETAL SYSTEM OF MALE ATHLETES

Yulia V. Koryagina^{1✉}, Julia V. Kushnareva¹, Sergey V. Nopin¹, Sabina M. Abutalimova¹, Igor N. Mitin²¹ North Caucasian Federal Scientific and Clinical Center, Yessentuki, Russia² National Center of Sports Medicine of the Federal Medical and Biological Agency, Moscow, Russia

Introduction. Previous research has demonstrated the high efficacy of peripheral magnetic stimulation (PMS) in the postoperative period of athletes for increasing strength and muscle tissue hypertrophy. Scientific and experimental substantiation for the use of PMS within the medical and biological support system for elite sports to improve the functional capabilities of the musculoskeletal system (MSS) in healthy athletes represents a relevant research task.

Objective. Study of the effect of rhythmic PMS on the functional capabilities of MSS in male athletes.

Materials and methods. The study was conducted at the Yug-Sport Rehabilitation and Recovery Center for Athletes in Kislovodsk (Russia). In total, 38 highly qualified male athletes were involved, who were divided into three groups: experimental group 1 (EG1) — 15 individuals, experimental group 2 (EG2) — 8 individuals, and the control group (CG) — 15 individuals. PMS was administered using a BTL-6000 Super Inductive System high-intensity magnetotherapy device, following two protocols for modulation and stimulation frequency. In the athletes, the following parameters were assessed: electrophysiological studies (electroneuromyography), rheovasography, and dynamometry of the lower limbs. These assessments were conducted before the intervention, after the first session, and after a course of eight PMS treatment sessions. In the control group, PMS was not applied. All parameters in this group were measured before and after the training camp period.

Results. The application of PMS to the posterior aspect of the left and right thigh at an intensity above the motor threshold (25–80%) and a frequency modulation of 1–150 Hz (EG1) contributed to a decrease in latency parameters and an increase in motor conduction velocity, as well as the M response amplitude and area upon stimulation of the peroneal nerve. The application of PMS to the same area and at the same intensity, but with a frequency modulation of 1–50 Hz (EG2), increased the M response amplitude and area. An increase in peripheral hemodynamics in the vessels of the lower limbs and in the strength parameters of the hip flexor and extensor muscles was revealed, along with an improvement in intermuscular coordination.

Conclusions. The application of PMS in highly qualified athletes contributes to an increase in the functional capabilities of MSS, manifested in improved parameters of neuromuscular transmission, peripheral hemodynamics, and strength capabilities.

Keywords: athletes; functional capacity; musculoskeletal system; neuromuscular system; peripheral magnetic stimulation; physiotherapy; electroneuromyography; hemodynamics; muscle strength

For citation: Koryagina Y.V., Kushnareva J.V., Nopin S.V., Abutalimova S.M., Mitin I.N. Effect of rhythmic peripheral magnetic stimulation on the functional capabilities of the musculoskeletal system of male athletes. *Extreme Medicine*. 2026;28(1):88–94. <https://doi.org/10.47183/mes.2025-367>

Funding: the study was conducted in accordance with contract No. 31V/CSM/23 dated 07.07.2023, for the execution of a component of applied research work on the topic: “Development and Scientific-Experimental Substantiation of the Application of Peripheral Magnetic Stimulation in the Medical-Biological Support System of Elite Sports for the Recovery and Improvement of the Functional State of the Neuromuscular Apparatus in Athletes” (code: “Magnet-23”).

Compliance with the ethical principles: the study was conducted in accordance with the ethical principles for medical research involving human subjects outlined in the 2013 Helsinki Declaration. The study was approved by the Local Ethical Committee for the Review of Biomedical Research at the North Caucasian Federal Research and Clinical Center of the FMBA of Russia (Minutes No. 2, of 30.06.2023). All participants provided written informed consent to participate in the study, as well as permission for the processing of their personal data.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Yulia V. Koryagina nauka@skfmba.ru

Received: 4 Aug. 2025 **Revised:** 6 Oct. 2025 **Accepted:** 31 Oct. 2025 **Online first:** 17 Dec. 2025

УДК 615.8

ВЛИЯНИЕ ПРИМЕНЕНИЯ РИТМИЧЕСКОЙ ПЕРИФЕРИЧЕСКОЙ МАГНИТНОЙ СТИМУЛЯЦИИ НА ФУНКЦИОНАЛЬНЫЕ ВОЗМОЖНОСТИ ОПОРНО-ДВИГАТЕЛЬНОГО АППАРАТА СПОРТСМЕНОВ-МУЖЧИН

Ю.В. Корягина^{1✉}, Ю.В. Кушнарева¹, С.В. Нопин¹, С.М. Абуталимова¹, И.Н. Митин²¹ Северо-Кавказский федеральный научно-клинический центр Федерального медико-биологического агентства, Ессентуки, Россия² Национальный центр спортивной медицины Федерального медико-биологического агентства, Москва, Россия

Введение. Исследования, проведенные специалистами, показали высокую эффективность применения периферической магнитной стимуляции (ПМС) в послеоперационном периоде для повышения силы и гипертрофии мышечной ткани у спортсменов. Актуальным является научно-экспериментальное обоснование применения ПМС в системе медико-биологического обеспечения спорта высших достижений для улучшения функциональных возможностей опорно-двигательного аппарата (ОДА) здоровых спортсменов.

© Y.V. Koryagina, J.V. Kushnareva, S.V. Nopin, S.M. Abutalimova, I.N. Mitin, 2025

Цель. Изучение влияния применения ритмической ПМС на функциональные возможности ОДА спортсменов-мужчин.

Материалы и методы. Исследование проведено на базе реабилитационно-восстановительного центра для спортсменов ФГБУ «Юг Спорт» в г. Кисловодске. В нем приняли участие 38 спортсменов-мужчин высокой квалификации. Спортсмены были разделены на три группы: опытную группу 1 (ОГ1) — 15 чел., опытную группу 2 (ОГ2) — 8 чел. и контрольную (КГ) — 15 чел. ПМС проводили с помощью системы высокочастотной магнитотерапии BTL-6000 Super Inductive System по двум разным протоколам модуляции и частоты воздействий. До применения, после первого сеанса и после курса из 8 процедур ПМС у спортсменов определялись показатели электромиографии, реовазографии и динамометрии нижних конечностей. В контрольной группе спортсменов ПМС не применяли, все показатели исследовали до и после учебно-тренировочных сборов.

Результаты. Применение ПМС на область задней поверхности левого и правого бедра с интенсивностью выше порога моторного ответа (25–80%) и модуляцией частоты 1–150 Гц (ОГ 1) способствовало снижению показателей латентности и увеличению параметров скорости моторного проведения, амплитуды и площади М-ответов при стимуляции малоберцового нерва. Применение ПМС на ту же область и с той же интенсивностью с модуляцией частоты 1–50 Гц (ОГ2) увеличивало показатели амплитуды и площади М-ответов. Выявлено увеличение периферической гемодинамики в сосудах нижних конечностей и параметров силы мышц-сгибателей и разгибателей бедра, улучшение межмышечной координации работы мышц.

Выводы. Применение ПМС у спортсменов высокой квалификации способствует увеличению функциональных возможностей ОДА: параметров нервно-мышечной передачи, периферической гемодинамики и силовых возможностей.

Ключевые слова: спортсмены; функциональные возможности; опорно-двигательный аппарат; нервно-мышечный аппарат; периферическая магнитная стимуляция; физиотерапия; электромиография; гемодинамика; сила мышц

Для цитирования: Корягина Ю.В., Кушнарева Ю.В., Нопин С.В., Абуталимова С.М., Митин И.Н. Влияние применения ритмической периферической магнитной стимуляции на функциональные возможности опорно-двигательного аппарата спортсменов-мужчин. *Медицина экстремальных ситуаций*. 2026;28(1):88–94. <https://doi.org/10.47183/mes.2025-367>

Финансирование: исследование проведено в соответствии с контрактом № 31В/ЦСМ/23 от 07.06.2023 на выполнение составной части прикладной научно-исследовательской работы по теме: «Разработка и научно-экспериментальное обоснование применения периферической магнитной стимуляции в системе медико-биологического обеспечения спорта высших достижений для восстановления и улучшения функционального состояния нейромышечного аппарата спортсменов» (шифр: «Магнит-23»).

Соответствие принципам этики: исследование проводилось в соответствии с этическими принципами медицинских исследований с участием человека, изложенными в Хельсинкской декларации 2013 г. Исследование было одобрено локальным этическим комитетом по экспертизе биомедицинских исследований ФГБУ СКФНЦФ ФМБА России (протокол № 2 от 30.06.2023). Все участники подписали информированное согласие на участие в исследовании, а также разрешение на обработку персональных данных.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Корягина Юлия Владиславовна nauka@skfmba.ru

Статья поступила: 04.08.2025 **После доработки:** 06.10.2025 **Принята к публикации:** 31.10.2025 **Online first:** 17.12.2025

INTRODUCTION

Magnetic stimulation is a highly effective treatment method widely used in clinical practice for the therapy and rehabilitation of patients with pathologies of the central and peripheral nervous systems, musculoskeletal system (MSS), and urological diseases [1–3]. In recent years, an increasing number of studies have been published investigating the effects of transcranial and peripheral rhythmic magnetic field exposure on the functional state of organs and systems in athletes [4, 5]. Research results have demonstrated the high efficacy of using peripheral magnetic stimulation (PMS) in the post-operative period to enhance strength and muscle tissue hypertrophy in athletes [6]. Data indicating increased muscle strength performance following a course of PMS have been published [7, 8]. Furthermore, by regulating the frequency characteristics of the magnetic field, it is possible not only to increase strength parameters but also to potentiate the aerobic capacity of the muscular system in athletes [9].

Therefore, scientific and experimental substantiation for the application of PMS within the medical and biological support system of elite sports to enhance the functional capabilities of the MSS in healthy athletes appears a highly relevant research task. In this article, we

aim to study the effect of rhythmic PMS on the functional capabilities of MSS in male athletes.

MATERIALS AND METHODS

The study was conducted at the rehabilitation and recovery center for Russian national team athletes, located on the premises of the Yug-Sport center (Kislovodsk, Russia). The study involved 38 highly qualified male athletes engaged in athletics, baseball, cross-country skiing, short track speed skating, kettlebell lifting, and badminton, with a median age of 22 [19; 25] years. The athletes were divided into three groups: experimental group 1 (EG1) — 15 individuals, experimental group 2 (EG2) — 8 individuals, and the control group (CG) — 15 individuals:

- in EG1, the pre-set protocol (A-1012) “Pre-Rehabilitation Preparation” of the device was applied (see Table 1);
- in EG2, the pre-set protocol (A-0012) “Muscle Strengthening” of the device was applied;
- in the CG, PMS was not applied; all parameters were assessed before and after the training camp period.

The inclusion criteria for the study were highly qualified athletes undergoing intensive training loads. The exclusion criteria were refusal to participate in the

study, acute illnesses and injuries, presence of contraindications to PMS (general contraindications to physiotherapy, arterial hypotension, clinically significant endocrinopathies (especially hyperthyroidism), bleeding tendency, hemorrhagic diathesis, presence of an implanted pacemaker).

PMS was performed using a BTL-6000 Super Inductive System high-intensity magnetotherapy device, specifically the BTL-6000 Super Inductive System Elite version (BTL Industries Ltd., the UK). The treatment session was conducted with the participant in a prone position, with the center of the applicator positioned at the minimal distance from the skin surface. The stimulation area targeted the posterior aspect of the left and right thigh (corresponding to the projection of the *m. biceps femoris* long head and *m. semimembranosus*). The stimulation intensity was set individually above the motor threshold (25–80%). The total number of sessions was 8, conducted daily.

Before and after the first session, as well as after the course of eight PMS sessions, the following parameters were recorded:

- motor response parameters using stimulation electroneuromyography (ENMG) by a four-channel Neuro-MVP complex (Neurosoft, Russia);
- peripheral hemodynamics via rheovasography (RVG) using a Valent rheography device;
- strength parameters of the thigh muscles via dynamometry using a robotic CON-TREX LP complex (Physiomed Elektromedizin AG).

A movement template (bilateral: extension/flexion, sitting position) was used during the measurement.

Statistical data processing was performed using the Statistica 13.0 software. Standard descriptive statistics measures were calculated (medians (M_0) with upper (Q_1) and lower (Q_3) quartiles). Comparison of parameters was

carried out using non-parametric tests: Wilcoxon test for analyzing data dynamics in dependent groups, Dunn's test for assessing differences between two independent groups, Kruskal–Wallis test for comparisons between three independent groups.

RESULTS

During the study, when comparing the parameters of the neuromuscular apparatus (NMA) based on ENMG data before PMS application in athletes of EG1, EG2, and CG using the Kruskal–Wallis and Dunn tests, statistically significant differences were found only for the duration and latency of the M response, which were higher in the athletes of the CG ($p < 0.05$).

The conducted analysis of the dynamics of motor response parameters during PMS in athletes of EG1 showed a statistically significant increase in:

- motor conduction velocity at the stimulation point “fibular head” on the right side after the PMS course (dynamics: pre-PMS 49.3 [47.7; 50.5] m/s, after the first session 50.3 [48.3; 51.4] m/s, after the PMS course 50 [48.7; 52] m/s ($p < 0.03$));
- motor conduction velocity at the stimulation point “popliteal fossa” on the left ($p < 0.02$);
- M-wave amplitude at the stimulation points “fibular head” ($p < 0.05$) and “popliteal fossa” on the left ($p < 0.03$);
- a decrease in latency at the stimulation point “tarsus” on the left ($p < 0.02$) (see Table 2).

A trend toward an increase in the M-wave area was noted at all stimulation points, both on the right and left sides.

The conducted analysis of the results of PMS application in athletes of EG2 showed a statistically significant increase in:

Table 1. Peripheral magnetic stimulation protocol parameters

PMS modes	Groups	
	EG1	EG2
Frequency modulation, Hz	1–150	1–50
Sequence, frequency (Hz), and duration (s) of frequency modulation sections	1. Variable (150 Hz), 30 s 2. No modulation (0 Hz), 30 s 3. No modulation (150 Hz), 15 s 4. Variable (1 Hz), 45 s 5. No modulation (150 Hz), 15 s 6. No modulation (1 Hz), 45 s 7. Trapezoidal (25–45 Hz), 240 s 8. No modulation (1 Hz), 60 s 9. Variable (50 Hz), 30 s 10. Variable (1 Hz), 30 s	1. Variable (15–50 Hz), 30 s 2. No modulation (1 Hz), 30 s 3. Trapezoidal (1–15 Hz), 120 s 4. No modulation (1 Hz), 60 s 5. Trapezoidal (15–30 Hz), 120 s 6. No modulation (1 Hz), 60 s 7. Trapezoidal (30–40 Hz), 120 s 8. No modulation (1 Hz), 60 s 9. Trapezoidal (40–50 Hz), 120 s 10. No modulation (1 Hz), 60 s
Procedure duration	10 min per zone	13 min per zone

Table compiled by the authors based on original data

Note: PMS — peripheral magnetic stimulation; EG1 — experimental group 1; EG2 — experimental group 2.

Table 2. Parameters of the motor response from the *m. extensor digitorum brevis* (by the left *n. peroneus*) in athletes of experimental group 1 during peripheral magnetic stimulation, $n = 15$

Parameters	Normal value	Before the 1 st session	PMS	
			After the 1 st session	After the course
Stimulation point “Tarsus”				
Terminal latency, ms	–	3.7 [3.5; 3.9]	3.6 [3.2; 3.9]†	3.5 [3.1; 4.3]†
Amplitude, mV	>3	8.5 [5.2; 10.4]	7.9 [5.6; 10.1]	8.4 [7.3; 11.7]
Duration, ms	–	5.95 [5.55; 6.35]	5.9 [5.5; 6.45]	5.82 [5.65; 6.7]
Area, mV × ms	–	22.4 [15.6; 32]	23.1 [19.6; 27.9]	26.9 [22.8; 33.7]
Residual latency, ms	<3	2.37 [2.01; 2.57]	2.27 [1.74; 2.51]	1.99 [1.79; 2.77]**
Stimulation point “Fibular head”				
Terminal latency, ms	–	11 [10.2;11.4]	10.8 [10; 11.1]	10.8 [9.9; 11.1]
Amplitude, mV	>3	7.5 [6; 10.4]	8.3 [5.1; 9.9]	7.9 [7.1; 11]*
Duration, ms	–	6.42 [5.9; 7.1]	6.38 [6.15; 7.05]	6.45 [5.9; 6.9]
Area, mV × ms	–	25.2 [19.9; 31.8]	23.8 [19.6; 32.7]	27.8 [21.5; 33.6]
Velocity, m/s	>40	48.8 [46.7; 50.7]	50 [48.3; 51.7]	49.6 [47.7; 51.3]
Stimulation point “Popliteal fossa”				
Terminal latency, ms	–	12.8 [12.5; 13.5]	12.7 [12; 13.2]*	12.7 [11.9; 12.9]
Amplitude, mV	>3	7.9 [6.2; 10.4]	8 [5.8; 10.2]	8.4 [7; 11.8]**
Duration, ms	–	6.7 [5.83; 7.11]	6.45 [5.97; 7.4]	6.5 [5.86; 7.57]
Area, mV × ms	–	27.7 [19.5; 33.7]	24.5 [19.2; 32.5]	28.6 [22.4; 37.5]
Velocity, m/s	>40	51.4 [48.3; 55.6]	52.6 [48.8; 55.6]	56.4 [51.2; 58.9]**

Table compiled by the authors based on original data

Note: PMS — peripheral magnetic stimulation; data are presented as median with lower and upper quartiles $M_e [Q_1; Q_3]$; n — number of athletes in the group; p — statistically significant differences from the parameter before the 1st session; * — $p < 0.05$; † — $p < 0.03$; ** — $p < 0.02$; “–” — data not available.

- M-wave amplitude at the stimulation points “tarsus” ($p < 0.03$), “fibular head” ($p < 0.04$), and “popliteal fossa” ($p < 0.04$) on the right side (Fig. 1A);
- M-wave area at the stimulation points “tarsus” ($p < 0.03$), “fibular head” ($p < 0.01$), and “popliteal fossa” ($p < 0.03$) on the right side (Fig. 1B).

A similar increase in the parameters of M response amplitude and area were observed in athletes of EG2 on the left side at all stimulation points. In the CG, a decrease in motor conduction velocity was observed at the stimulation point “popliteal fossa” on the left side (before the training camp — 54 [51.2; 57.8] m/s, after — 48.1 [44.7; 52.8] m/s; $p < 0.02$).

The comparison of RVG parameters before PMS application in athletes of EG1, EG2, and CG revealed statistically significant differences only in the elasticity modulus (EM) parameters, which were higher in the CG ($p < 0.04$). The analysis of lower limb hemodynamic parameters in athletes of EG1 showed a statistically

significant increase after the first session in the “left foot” segment for the following parameters:

- rheographic index 1.4 [0.97; 1.71] conv. units ($p < 0.05$) compared to pre-PMS values of 1.13 [0.93; 1.29] conv. units and post-course PMS values of 1.32 [1.07; 1.49] conv. units;
- propagation time of rheographic waves pre-PMS 0.3 [0.28; 0.3] s; after the first session 0.32 [0.3; 0.32] s ($p < 0.006$); post-course 0.3 [0.29; 0.31] s.

In the “right foot” segment, the duration of maximum systolic vessel filling increased: pre-PMS 0.13 [0.115; 0.142] s; after the first session 0.142 [0.132; 0.154] s ($p < 0.05$); post-course 0.119 [0.107; 0.15] s.

In the “left shin” segment, after the first session, the propagation time of rheographic waves increased to 0.28 [0.26; 0.29] s ($p < 0.05$) against the background of pre-PMS values of 0.27 [0.26; 0.28] s and post-therapy course values of 0.27 [0.26; 0.29] s.

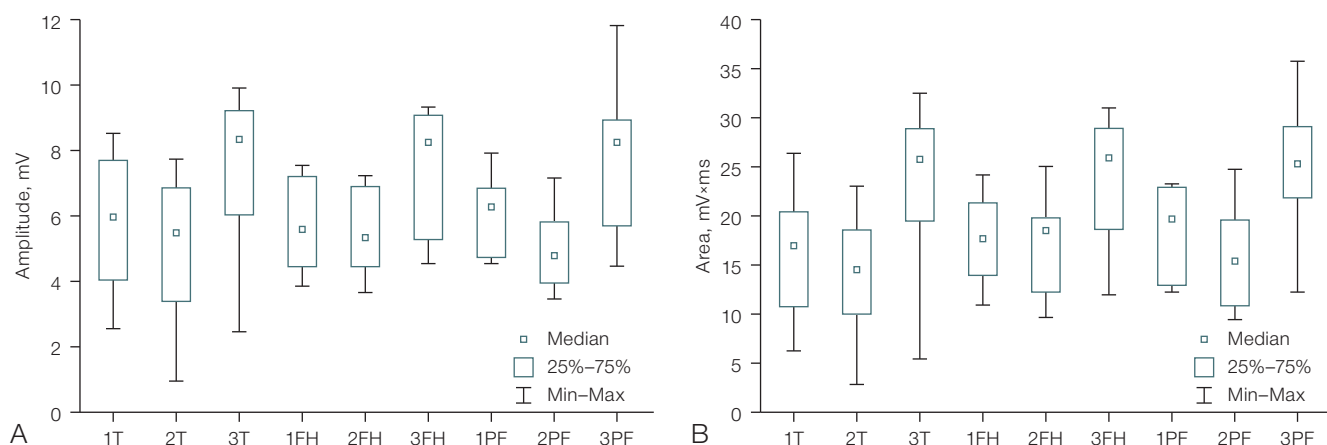


Figure prepared by the authors based on original data

Fig. 1. Dynamics of amplitude (A) and area (B) of the M response at the stimulation points “tarsus”, “fibular head”, and “popliteal fossa” (*n. peroneus*) in athletes of experimental group 2 during peripheral magnetic stimulation, $n = 8$: n — number of athletes in the group; T — tarsus; FH — fibular head; PF — popliteal fossa; 1 — before the 1st session; 2 — after the 1st session; 3 — after a course of peripheral magnetic stimulation

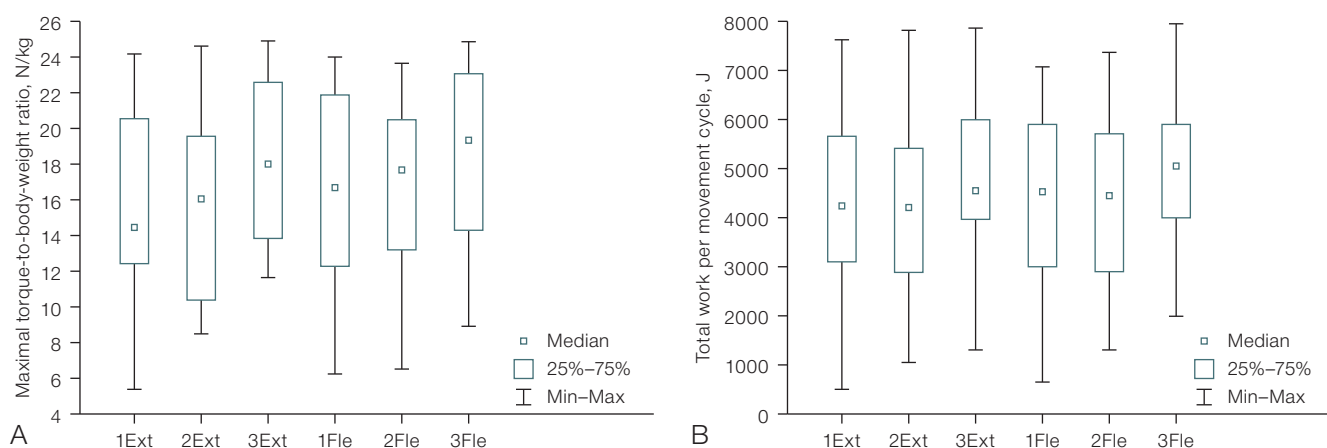


Figure prepared by the authors based on original data

Fig. 2. Dynamics of the mean maximal torque-to-body-weight ratio (A) and total work per movement cycle (B) in athletes of experimental group 1, $n = 15$: n — number of athletes in the group; Ext — extensor muscles; Fle — flexor muscles; 1 — before the 1st session; 2 — after the 1st session; 3 — after a course of peripheral magnetic stimulation

In the “right shin” segment, the diastolic index was higher both after the first session and after the entire course (pre-PMS — 0.43 [0.31; 0.46] conv. units; after the first session — 0.52 [0.44; 0.61] conv. units, $p < 0.04$; after the course — 0.5 [0.36; 0.59] conv. units, $p < 0.04$).

After the full PMS course, the blood flow asymmetry coefficient in the feet decreased threefold (pre-PMS — 34 [12; 56]; after the 1st session — 13 [7; 27]; after the course — 11 [4; 24], $p < 0.03$). In the CG athletes, the diastolic index decreased statistically significantly in the “left foot” segment (pre-training camp — 0.47 [0.39; 0.53] conv. units and post-training camp — 0.38 [0.32; 0.44] conv. units, $p < 0.02$) and the “right foot” segment (pre-training camp — 0.46 [0.41; 0.56] conv. units and post-training camp — 0.38 [0.36; 0.45] conv. units, $p < 0.05$).

The comparison of dynamometry parameters in athletes of EG1 and CG before the application of PMS

revealed no statistically significant differences. The analysis of the dynamics of thigh muscle strength in men of EG1 showed a statistically significant increase in the strength parameters of the hip extensor and flexor muscles after completing the entire course. Specifically, for the extensor muscles, the following parameters increased:

- maximum torque strength, in newtons (N): pre-PMS — 1475 [1277; 1714] N; after the first session — 1442.5 [1034; 1629] N; after the course — 1610 [1277; 2018] N, $p < 0.05$;
- mean maximal torque-to-body-weight ratio, N: pre-PMS — 14.45 [12.4; 20.52] N; after the first session — 16.01 [10.39; 19.53] N; after the course — 17.97 [13.84; 22.56] N, $p < 0.03$ (Fig. 2A).

In flexor muscles, a statistically significant increase in the following parameters was observed:

- mean maximal power per movement cycle, N: before PMS — 128.3 [91; 163] N; after the first

session — 116.2 [83.2; 170.9] N; after the course — 140.1 [117.1; 175.5] N; $p < 0.04$;

- mean work per cycle (per movement direction), normalized to body weight, N: before PMS — 226 [150.5; 293] N; after the first session — 221.95 [144.7; 284.3] N; after the course — 252.2 [199.6; 294.2] N; $p < 0.05$;
- total work per movement cycle, N: before PMS — 4519.1 [3010.4; 5860.6] N; after the first session — 4438.7 [2894.6; 5686.2] N; after the course — 5044.5 [3992.8; 5883.6] N; $p < 0.05$ (Fig. 2B).

Additionally, after both the single session and the full PMS course, athletes in EG1 showed optimization of extensor and flexor muscle strength. This was reflected in a decrease in the ratio of this parameter (before PMS — 109.6 [101; 123.6]%; after the first session — 105.15 [94.6; 107.7]%; $p < 0.05$; after the course — 103.5 [99.3; 112.2]%; $p < 0.04$). The analysis of dynamometry parameters of the thigh muscles in the CG revealed no statistically significant differences.

DISCUSSION

The application of PMS in clinical practice is widely documented in the scientific literature, particularly in the treatment of myofascial pain syndrome, other MSS disorders, patients with peripheral neuropathic pain, acute and chronic back pain, post-stroke spasticity, paresis, plegia, dysphagia [10–14]. In sports practice, the use of rhythmic PMS is most frequently reported in studies focusing on methods to enhance athlete performance. For instance, applying rhythmic PMS in athletes during isokinetic exercises revealed an increase in muscle aerobic capacity, attributed to higher oxygen uptake at the aerobic and anaerobic thresholds. Additionally, an improvement in muscle strength parameters was observed without an increase in muscle mass [9].

Our study has found that a course of eight PMS treatment sessions applied to the posterior surface of both left and right thighs — using intensities above the motor response threshold (25–80 %) and frequency modulation of 1–150 Hz — led to:

- a reduction in latency values;
- an increase in motor conduction velocity;
- higher amplitude and area of M responses during peroneal nerve stimulation.

A PMS course applied to the same region at the same intensity, but with a frequency modulation of 1–50 Hz, resulted in increased amplitude and area of

M responses. These changes indicated the attainment of potentially greater NMA capabilities in improvement of intramuscular coordination mechanisms, activation and synchronization of motor units. This, in turn, contributed to a greater expression of strength capabilities. Dynamometry assessments revealed that athletes exhibited a statistically significant increase in all measured strength parameters of both hip flexor and extensor muscles. Additionally, intermuscular coordination of their activity improved.

The results obtained complement previously published data demonstrating that 10 sessions of PMS applied to the *m. gastrocnemius* during its voluntary contraction in healthy men led to a greater increase in maximal torque (muscle force) and an enhancement of H reflex (Hoffmann's reflex) amplitude [15]. The authors suggest that one mechanism underlying the improvement in strength parameters is an increase in reflex excitability of the corresponding spinal motor neuron pool. The recorded higher strength values result from additional activation of high threshold (fast) motor units [15–17].

Current research identifies enhanced microcirculation in muscle tissue as one of the key mechanisms underlying increased muscle strength. This improvement creates optimal conditions for altering the concentration of potassium, sodium, and calcium ions across semi-permeable cell membranes, thereby facilitating depolarization processes and the generation of action potentials [16]. Our study has shown that PMS application enhanced blood flow in athletes. Overall, a review of scientific literature suggests that the applied PMS modulation and frequency parameters contribute to the activation of specific physiological processes, including enhanced metabolic reactions, modulation of neuroplasticity, improved cellular permeability. Collectively, these effects improve the functional state of MSS in athletes.

CONCLUSION

Our study of the effects of rhythmic PMS on the functional capabilities of MSS in male athletes demonstrated that its application with pre-set modulation and frequency parameters leads to statistically significant improvements in neuromuscular transmission parameters, peripheral hemodynamics, and strength capabilities. Therefore, this method can be recommended for inclusion in the biomedical support of athletes to enhance the functional state of MSS and increase strength capabilities.

References

1. Pomytkin AN, Lebedeva IS, Tikhonov DV, Kaleda VG. Rhythmic transcranial magnetic stimulation in the treatment of resistant depression in schizophrenia. *S.S. Korsakov Journal of Neurology and Psychiatry*. 2021;121(5–2):99–105 (In Russ.).
<https://doi.org/10.17116/jnevro202112105299>
2. Novikova ES, Rozanov ID. Low-dose radiotherapy for chronic pain syndrome in specific and non-specific diseases of the musculoskeletal system. *Medical Alphabet*. 2019;1(2):58 (In Russ.).
EDN: [VWLIRK](#)
3. Pushkar DY, Kulikov AG, Kasyan GR, Kupriyanov YA, Romikh VV, Zakharchenko AV, et al. Extracorporeal magnetic stimulation of the pelvic floor neuromuscular system

- in urological practice. *Russian Journal of Physiotherapy, Balneology and Rehabilitation*. 2019;18(4):264–76 (In Russ.). <https://doi.org/10.17816/1681-3456-2019-18-4-264-276>
4. Pospelova ML, Kasumova AA, Fionik OV, Alekseeva TM, Samochernykh KA, Krasnikova VV. Possibilities of using the transcranial magnetic stimulation method in the treatment of chronic pain syndromes. *Modern Problems of Science and Education*. 2021;2:195 (In Russ.). <https://doi.org/10.17513/spno.30631>
 5. Kutashov VA, Ul'yanova OV. Application of transcranial magnetic stimulation with in complex treatment of patients with ischemic stroke in the late recovery period from medical rehabilitation positions. *Herald of Physiotherapy and Health Resort Therapy*. 2018;24(3):73–80 (In Russ.).
 6. Abulhasan JF, Rumble YL, Morgan ER, Slatter WH, Grey MJ. Peripheral electrical and magnetic stimulation to augment resistance training. *Journal of Functional Morphology and Kinesiology*. 2016;1(3):328–42. <https://doi.org/10.3390/jfmk1030328>
 7. Gorodnichev RM, Belyaev AG, Pivovarova EA, Shlyakhtov VN. The effect of electromagnetic stimulation on muscle strength indices. *Human Physiology*. 2014;40(1):76 (In Russ.). <https://doi.org/10.7868/s013116461304005x>
 8. Jia Y, Liu X, Wei J, Li D, Wang C, Wang X, et al. Modulation of the corticomotor excitability by repetitive peripheral magnetic stimulation on the median nerve in healthy subjects. *Frontiers in Neural Circuits*. 2021;15:616084. <https://doi.org/10.3389/fncir.2021.616084>
 9. Popov GI, Malkhasyan EA, Markaryan VS, Kalinin EM, Seluyanov VN. Influence of rhythmical magnetic stimulation as a way of increase in aerobic possibilities of muscles. *Lomonosov Journal of Anthropol.* 2013;2:106–13 (In Russ.). EDN: QZDRGH
 10. Merkulov YA, Gorelikov AE, Pyatkov AA, Merkulova DM. Repetitive transspinal magnetic stimulation in the treatment of chronic low back pain. A meta-analysis (Part II). *Pathological Physiology and Experimental Therapy*. 2021;65(4):97–108 (In Russ.). <https://doi.org/10.25557/0031-2991.2021.04.97-108>
 11. Blokhina VN, Kuznetsov AN, Vinogradov OI, Voytenkov VB, Melikyan EG, Nikolayev SG. Comparative analysis of excitability of radicular and intramuscular axonal systems in healthy volunteers during peripheral magnetic stimulation. *Neuromuscular Diseases*. 2017;7(2):48–53 (In Russ.). <https://doi.org/10.17650/2222-8721-2017-7-2-48-53>
 12. Anoshina EA, Mikhaylenko AA, Samartsev IN, Zhivolupov SA. Analysis of the effectiveness of analgesics effects of physiotherapeutics and interventional treatment methods for a number of the most common pain syndromes in neurological practice. *Russian Military Medical Academy Reports*. 2019;S3:16–8 (In Russ.).
 13. Zhivolupov SA, Samartsev IN, Kovalenko AP. Medical rehabilitation in diseases and injuries of the nervous system. *Victims in Emergency Situations*. New York: Xlibris LLC; 2014 (In Russ.).
 14. Gorelikov AE, Melnikova EA, Rud IM. Magnetic Stimulation in the Treatment and Rehabilitation of Patients with Nervous System and Spinal Disorders. *Doctor.Ru*. 2017;11(140):46–50 (In Russ.).
 15. Gorodnichev RM, Belyaev AG, Pivovarova EA, Shlyakhtov VN. The effect of electromagnetic stimulation on the parameters of muscular strength. *Human Physiology*. 2014;40(1):76 (In Russ.). <https://doi.org/10.7868/S013116461304005X>
 16. Kamo T, Wada Y, Okamura M, Sakai K, Momosaki R, Taito S. Repetitive peripheral magnetic stimulation for impairment and disability in people after stroke. *Cochrane Database of Systematic Reviews*. 2022;9(9):CD011968. <https://doi.org/10.1002/14651858.CD011968.pub4>
 17. Fujimura K, Kagaya H, Endou C, Ishihara A, Nishigaya K, Muroguchi K, et al. Effects of repetitive peripheral magnetic stimulation on shoulder subluxations caused by stroke: A preliminary study. *Neuromodulation: Technology at the Neural Interface*. 2020;23(6):847–51. <https://doi.org/10.1111/ner.13064>

Authors' contributions. All authors confirm that their authorship complies with the ICMJE criteria. The main contribution is distributed as follows: Yulia V. Koryagina — supervision during planning and conducting the study, critical analysis of the draft manuscript, making comments and corrections, critical analysis of the work for scientific novelty; Julia V. Kushnareva — collection of experimental data on the PMS use in athletes and source tables preparation; Sergey V. Nopin — data interpretation, study design, final approval of the manuscript; Sabina M. Abutalimova — manuscript drafting; Igor N. Mitin — collection of experimental data on the PMS use in athletes.

AUTHORS

Yulia V. Koryagina, Dr. Sci. (Biol.), Professor
<https://orcid.org/0000-0001-5468-0636>
nauka@skfmba.ru

Julia V. Kushnareva, Cand. Sci. (Biol.)
<https://orcid.org/0000-0002-7343-4622>
juli_83-83@mail.ru

Sergey V. Nopin, Dr. Sci. (Biol.)
<https://orcid.org/0000-0001-9406-4504>
work800@yandex.ru

Sabina M. Abutalimova, Cand. Sci. (Med.)
<https://orcid.org/0000-0003-1722-0774>
sabina190989@yandex.ru

Igor N. Mitin, Cand. Sci. (Med.)
<https://orcid.org/0000-0002-2168-921X>
pino4t@list.ru

<https://doi.org/10.47183/mes.2025-331>



ASSESSMENT OF THE RELATIONSHIP BETWEEN TRACHEAL BREATHING SOUNDS AND LUNG VENTILATION DURING PHYSICAL EXERCISE

Svetlana N. Astafyeva^{1✉}, Alexander I. Dyachenko¹, Irina A. Ruzhichko¹, Anatoly E. Kostiv²

¹ Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia

² Il'ichev Pacific Oceanological Institute of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia

Introduction. Assessment of the functional state of the respiratory system is a relevant task in the fields of sports, aerospace, and maritime medicine. Direct flowmetry methods cannot always be applied under conditions of a sealed enclosed environment. The recording and analysis of lung sounds appears to be a promising method for assessing the state of the respiratory system.

Objective. To assess the relationship between the amplitude characteristic of the recorded lung sound signal and the magnitude of pulmonary ventilation, as well as the applicability of the acoustic method for assessing the respiratory rate in healthy individuals during physical exercise, regardless of age and sex.

Materials and methods. The study involved 25 volunteers (20 male and 5 female) aged 23–59 years (mean age 35.5 ± 8.7 years). The participants were subjected to a stepwise increasing workload on an Ergoselect 200P cycle ergometer (Ergoline GmbH, Germany) up to sub-maximal heart rate levels, with simultaneous recording of respiratory sounds over the extrathoracic section of the trachea and measurement of respiratory flow via direct flowmetry using a Jaeger Oxycon Pro device. Statistical data processing was performed using the Statistica 13 software (StatSoft Inc., USA). To assess the relationship between respiratory sound power and pulmonary ventilation, a correlation analysis was conducted using Spearman's rank correlation coefficient (r_s).

Results. During the study, the achieved maximum power output for all participants ranged 105–240 W; only two subjects were capable of developing a power level exceeding 210 W. Dependencies of respiratory sound power on pulmonary ventilation were obtained. Spearman's rank correlation coefficient between the studied parameters was 0.58 ($p < 0.001$). Significant changes in the mean power of respiratory sounds were observed with an increase in load and pulmonary ventilation, already at the 30 W stage compared to the resting state (0 W) ($p < 0.0001$). The power of tracheal respiratory sounds also increased by 56% between the 120 W and 135 W load stages ($p = 0.023$) and by 75% between the 180 W and 195 W load stages ($p = 0.043$). No significant differences were found between respiratory rate assessments obtained by direct flowmetry and acoustic methods.

Conclusions. A statistically significant, moderate positive correlation was established between the magnitude of pulmonary ventilation and the mean power of respiratory sounds ($r_s = 0.58$; $p < 0.001$). For pulmonary ventilation values up to 60 L/min, the relationship between the mean power of tracheal sounds and pulmonary ventilation was found to be linear. A satisfactory agreement was determined between the acoustic assessment of respiratory rate and the data obtained by direct flowmetry methods. The analysis of respiratory sounds is capable of providing an indirect assessment of the state of the respiratory system.

Keywords: physical activity; lung sounds; cycle ergometry; pulmonary ventilation; respiratory system; respiratory acoustics

For citation: Astafyeva S.N., Dyachenko A.I., Ruzhichko I.A., Kostiv A.E. Assessment of the relationship between tracheal breathing sounds and lung ventilation during physical exercise. *Extreme Medicine*. 2026;28(1):95–101. <https://doi.org/10.47183/mes.2025-331>

Funding: the work was performed within the framework of the RAS (Russian Academy of Sciences) basic research theme FMFR-2024-0038.

Acknowledgments: the authors express their profound gratitude to the late Dr. Sci. (Engin.) Vladimir I. Korenbaum and Dr. Sci. (Med.) Alexander V. Suvorov for their invaluable consultations and assistance during the early stages of this research.

Compliance with the ethical principles: the study was conducted in accordance with the principles of the Helsinki Declaration. All volunteers underwent medical selection by the Medical Expert Commission of the SSC RF — IBMP RAS, during which no diseases or pathologies preventing participation in the experiment were identified. All participants provided signed voluntary informed consent to participate in the study. The study was approved by the Biomedical Ethics Committee of the SSC RF — IBMP RAS (Protocols No. 520 of 25.07.2019, and No. 539 of 17.03.2020).

Potential conflicts of interest: the authors declare no conflict of interest.

✉ Svetlana N. Astafyeva a10351@ya.ru

Received: 25 June 2025 **Revised:** 3 Sep. 2025 **Accepted:** 3 Oct. 2025 **Online first:** 31 Oct. 2025

УДК 612.216.1

ОЦЕНКА ЗАВИСИМОСТИ МОЩНОСТИ ТРАХЕАЛЬНЫХ ДЫХАТЕЛЬНЫХ ШУМОВ ОТ ЛЕГОЧНОЙ ВЕНТИЛЯЦИИ ПРИ ФИЗИЧЕСКОЙ НАГРУЗКЕ

С.Н. Астафьева^{1✉}, А.И. Дьяченко¹, И.А. Ружичко¹, А.Е. Костив²

¹ Институт медико-биологических проблем РАН, Москва, Россия

² Тихоокеанский океанологический институт им. В.И. Ильичева Дальневосточного отделения РАН, Владивосток, Россия

Введение. Оценка функционального состояния дыхательной системы является актуальной задачей в областях спортивной, космической и морской медицины. Использование методов прямой флоуметрии в условиях замкнутого гермообъекта не всегда возможно. Регистрация и анализ дыхательных шумов представляет перспективным способом оценки состояния респираторной системы.

© S.N. Astafyeva, A.I. Dyachenko, I.A. Ruzhichko, A.E. Kostiv, 2025

Цель. Выявление возможного характера зависимости между амплитудной характеристикой регистрируемого сигнала дыхательных шумов и величиной легочной вентиляции, а также степени применимости акустического метода для оценки частоты дыхания у здоровых людей при выполнении физической нагрузки независимо от возраста и пола.

Материалы и методы. В исследовании приняли участие 25 добровольцев (20 мужчин и 5 женщин) в возрасте 23–59 лет (средний возраст $35,5 \pm 8,7$ года). Обследуемые выполняли ступенчато-возрастающую нагрузку на велоэргометре Ergoselect 200P (Ergoline GmbH, Германия) до субмаксимальных величин частоты сердечных сокращений с одновременной регистрацией дыхательных шумов над внегрудным участком трахеи, а также величины дыхательного потока методом прямой флоуметрии на приборе Jaeger Oxycon Pro. Статистическая обработка данных проводилась с помощью программного обеспечения Statistica 13 (StatSoft Inc., США). Для оценки взаимосвязи мощности дыхательных шумов и вентиляции легких проведен корреляционный анализ с использованием коэффициента ранговой корреляции Спирмена (r_s).

Результаты. В ходе исследования у всех испытуемых достигнутые величины максимальной мощности находились в диапазоне 105–240 Вт; мощность свыше 210 Вт смогли развить только 2 испытуемых. Получены зависимости величины мощности шумов от легочной вентиляции. Коэффициент ранговой корреляции Спирмена между изучаемыми параметрами равен 0,58 ($p < 0,001$). Отмечены значимые изменения средней мощности дыхательных шумов при росте нагрузки и легочной вентиляции уже на ступени 30 Вт относительно состояния покоя (0 Вт) ($p < 0,0001$). Мощность трахеальных дыхательных шумов также увеличивалась на 56% между ступенями нагрузки 120 и 135 Вт ($p = 0,023$) и на 75% при нагрузке 180 и 195 Вт ($p = 0,043$). Значимых различий между оценками частоты дыхания методом прямой флоуметрии и акустическим способом не выявлено.

Выводы. Установлена статистически значимая умеренная положительная корреляционная взаимосвязь между величиной легочной вентиляции и средней мощностью дыхательных шумов ($r_s = 0,58$; $p < 0,001$). При значениях легочной вентиляции до 60 л/мин характер зависимости средней мощности трахеальных шумов от легочной вентиляции является линейным. Установлено удовлетворительное соответствие акустической оценки частоты дыхания данным, полученным методами прямой флоуметрии; анализ дыхательных шумов способен дать косвенную оценку состояния дыхательной системы.

Ключевые слова: физическая нагрузка; дыхательные шумы; велоэргометрия; легочная вентиляция; дыхательная система; респираторная акустика

Для цитирования: Астафьева С.Н., Дьяченко А.И., Ружичко И.А., Костив А.Е. Оценка зависимости мощности трахеальных дыхательных шумов от легочной вентиляции при физической нагрузке. *Медицина экстремальных ситуаций*. 2026;28(1):95–101. <https://doi.org/10.47183/mes.2025-331>

Финансирование: работа выполнена в рамках базовой темы РАН FMFR-2024-0038.

Благодарности: авторы выражают благодарность покойным д-ру тех. наук Коренбауму В.И. и д-ру мед. наук Суворову А.В. за ценные консультации и помощь на ранних этапах исследования.

Соответствие принципам этики: исследование проведено в соответствии с принципами Хельсинкской декларации. Все добровольцы прошли медицинский отбор врачебной экспертной комиссией ГНЦ РФ — ИМБП РАН, в ходе которого заболеваний и патологий, препятствующих участию в эксперименте, выявлено не было. Все участники подписали добровольное информированное согласие на участие в исследовании. Исследование одобрено Комиссией по биомедицинской этике ГНЦ РФ — ИМБП РАН (протоколы № 520 от 25.07.2019, № 539 от 17.03.2020).

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Астафьева Светлана Николаевна a10351@ya.ru

Статья поступила: 25.06.2025 **После доработки:** 03.09.2025 **Принята к публикации:** 03.10.2025 **Online first:** 31.10.2025

INTRODUCTION

In sports, aerospace, and maritime medicine, as well as in somnology studies, the issue of assessing the functional state of the human respiratory system is rather acute. This issue is addressed using the methods of direct flowmetry, which measure the magnitude of the respiratory flow. However, in a sealed enclosed environment (e.g., an orbital station or a spacesuit), direct flowmetry may not be possible or practical. In the search for a more compact and autonomous method for monitoring and assessing the state of the respiratory system, methods of respiratory acoustics are being investigated, which involve the recording and analysis of sounds generated during breathing [1–3].

The auscultation method is widely used in clinical medicine for an indirect assessment of pathological conditions of the lungs, at the same time as finding application in the field of maritime medicine [4, 5]. During spaceflight, including during physical exercise, monitoring the functional state of the respiratory organs to prevent

bronchopulmonary diseases is of primary importance. In such conditions, astronauts experience changes in the shape of the chest, a cranial shift of the diaphragm, and a decrease in the functional residual lung capacity [6–8]. These phenomena affect the dimensions of the airways, leading to changes in the acoustic characteristics of lung sounds.

In this study, we set out to establish the presence and nature of relationships between the amplitude characteristic of the recorded lung sound signal and the magnitude of ventilation during physical exercise in healthy human participants, regardless of age and gender. Furthermore, we analyze the applicability of the acoustic method for assessing the respiratory rate.

MATERIALS AND METHODS

The study involved 25 volunteers (20 males and 5 females) aged 23–59 years (mean age 35.5 ± 8.7 years). The height ranged 165–189 cm (mean 178 ± 6.6 cm), and body weight ranged 56–104 kg (mean 79.2 ± 11.3 kg). All

the participants were non-smokers and had no history of bronchopulmonary diseases. The volunteers underwent medical selection by an expert medical commission, during which no diseases or pathologies preventing participation in the experiment were identified. All the participants signed voluntary informed consent to participate in the study and for the use of their anonymized data.

The participants were subjected to a stepwise increase in physical workload on an Ergoselect 200P cycle ergometer (Ergoline GmbH, Germany). The initial workload stage was 30 W followed by a 15 W increase in each stage, which lasted for 1 min. The study was conducted until a submaximal heart rate¹ was reached under laboratory conditions with optimal ambient microclimate and atmospheric composition [9].

The participants breathed through a mouthpiece connected to the following pneumatic circuit: a tube with

a saliva trap; an individual disposable filter; the measurement unit of the Jaeger Oxycon Pro system (Germany), which includes a rotary air flow sensor (turbine) and tubes for sampling exhaled air for breath-by-breath gas analysis.

Lung sounds over the extrathoracic section of the trachea and the magnitude of the respiratory flow were continuously recorded by the direct flowmetry method using a Jaeger Oxycon Pro device. Based on the respiratory flow dynamics, the built-in device software determined the values of pulmonary ventilation and respiratory rate, which were further analyzed.

Recording of lung sounds was carried out using equipment comprising a lightweight accelerometer, a power supply unit, a voice recorder, and cables [10–12]. The PCB 333B52 accelerometer (USA) was placed over the extrathoracic section of the trachea on the subject's neck and secured to the skin surface using a Velcro

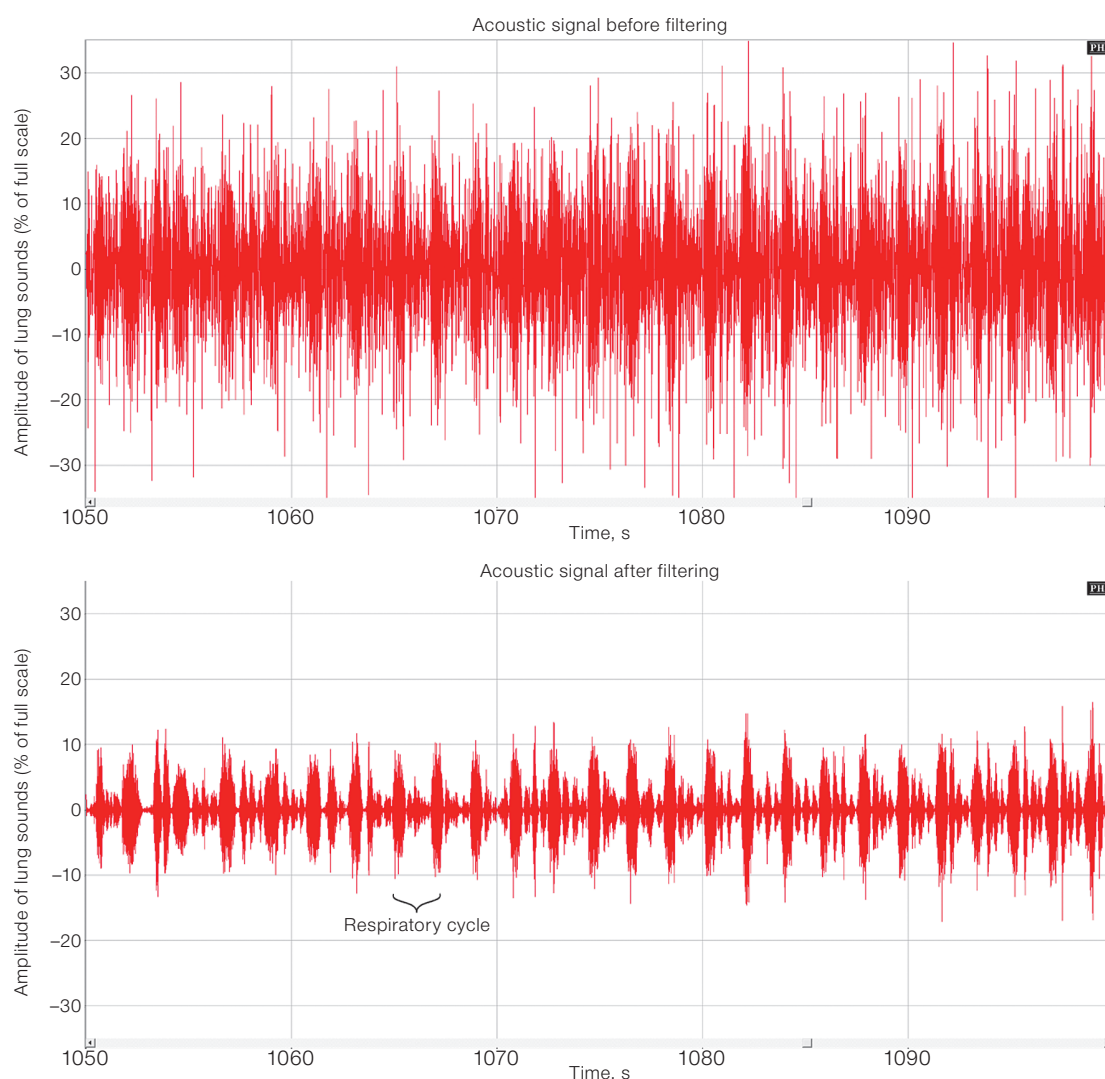


Figure prepared by the authors based on their own data

Fig. 1. Filtering of the lung sound signal: x-axis — time (sec); y-axis — amplitude of lung sounds (% of full scale)

¹ Bystrova AG, Kovaleva IO, Kuz'mina AYU, Parnes EYa, Potievsky BG, Erenburg IV, et al. Cycle ergometry in civil aviation aeromedical expertise. Manual. Moscow: RMANPO; 2020.

elastic strap. The accelerometer signal was digitized at a frequency of 48 kHz.

In order to attenuate background noise and the cardiac artifact, band-pass filtering was applied (Fig. 1). The acoustic signal was processed using an algorithm similar to that described in [11]. The lung sound recordings were processed by the SpectraPLUS 5.0 software (USA) using a band-pass filter in the frequency range of 200–1000 Hz to remove cardiac and muscle noises, which are typically observed at frequencies below 200 Hz, as well as high-frequency interference with frequencies above 1000 Hz [13].

The distance between adjacent peaks characterizing expiratory noises was considered the duration of the respiratory cycle, allowing an acoustic assessment of the respiratory rate (RR) and the period of the respiratory cycle. Furthermore, the SpectraPLUS software was used to determine the root mean square (RMS) value of the lung sound (RS) amplitude, taking into account each digitized value of the accelerometer signal corresponding to the load stages. This value is conventionally referred to as the mean power of lung sounds and is expressed as a percentage (%) of the full scale, i.e., the maximum signal amplitude value [11, 13–15].

Statistical data analysis was performed using the Statistica 13 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA) software packages. To assess the dynamics of the parameters, non-parametric analysis of variance (Friedman ANOVA) was applied; changes in the studied parameters were evaluated using the Wilcoxon test (for RS power and pulmonary ventilation) and the Mann–Whitney U test (for comparing the acoustic assessment of RR and flowmetry data). To assess the relationship between RS power and pulmonary ventilation, a correlation analysis was conducted using Spearman's

rank correlation coefficient (r_s) [16]. To establish the dependence of RS power on pulmonary ventilation, linear regression analysis was applied.

RESULTS AND DISCUSSION

During the study, the achieved maximum power output for all subjects ranged 105–240 W. However, only two individuals were capable of achieving a power output exceeding 210 W. In order to obtain more accurate data, the statistical analysis was limited to the power range of up to 210 W.

Dependencies of the mean lung sound (LS) power and the magnitude of pulmonary ventilation on the magnitude of physical load were obtained (Fig. 2).

A significant increase in the mean RS power from 0.0004 to 0.0009% was observed already at the 30 W stage compared to the resting state (0 W) ($p < 0.0001$). Furthermore, the power of lung sounds also increased by 56% between the 120 W and 135 W load stages ($p = 0.023$), and by 75% between the 180 W and 195 W load stages ($p = 0.043$).

The increase in RMS with an increase in physical load (Fig. 2) indicates a rise in the amplitude of lung sounds. The conducted correlation analysis established a statistically significant, moderate positive relationship between the absolute values of pulmonary ventilation and lung sound power ($r_s = 0.58$; $p < 0.001$).

The nature of the presumed relationship between RMS and the magnitude of pulmonary ventilation was analyzed by a linear regression analysis across the entire dataset (Fig. 3). Under an increase in the pulmonary ventilation volume, a scatter in the individual noise power values was observed. It appears that a single linear dependence cannot describe all individual parameters.

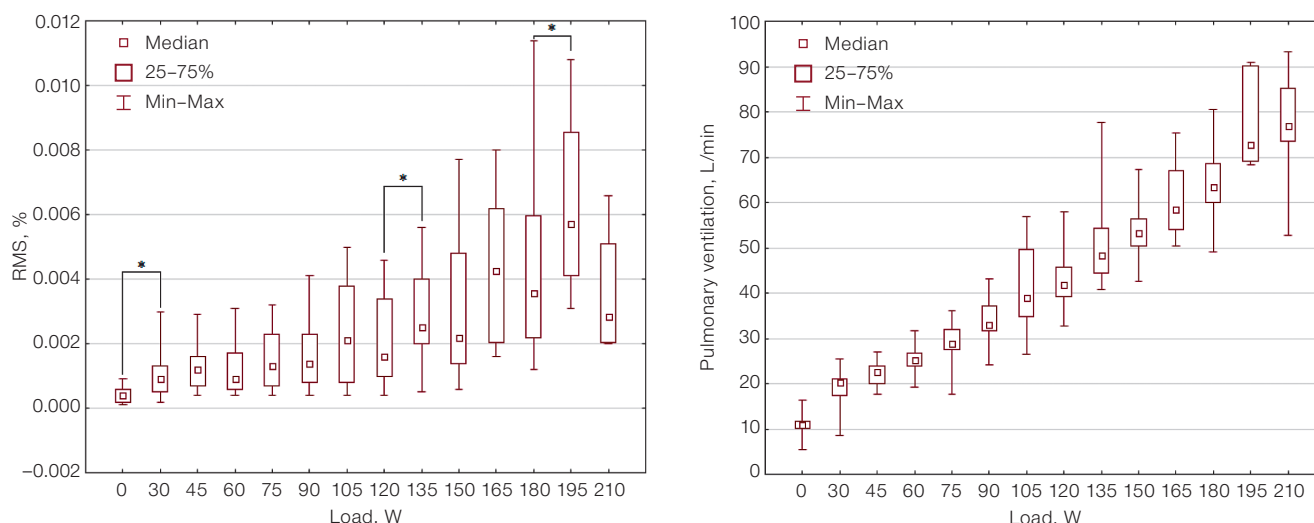


Figure prepared by the authors based on their own data

Fig. 2. Dependence of the mean lung sound power and pulmonary ventilation on the magnitude of stepwise increasing physical load

Note: RMS — root mean square value of the lung sound amplitude; * — statistically significant changes ($p < 0.05$).

Significant individual differences and the non-linearity of the relationship between noise power and pulmonary ventilation led to data scatter and a low coefficient of determination value of $R^2 = 0.35$.

Literature sources present various methods for describing the relationship between respiratory flow and the amplitude of lung sounds [17]. Banaszak et al. described a linear relationship between instantaneous flow and breath sound amplitude measured over the chest wall [18]. Shykoff et al. utilized a quadratic function [14, 19]. Gavriely et al. characterized a power-law function, with the group-averaged exponent being 1.66 ± 0.35 , which, in their view, is significantly less than the presumed second power [20, 21].

As noted above, the relationship between individual noise power values and pulmonary ventilation is characterized by a large scatter of noise power at a given level of pulmonary ventilation (Fig. 3). This scatter appears to be related to the significant differences in the geometry of the airway tree among the group participants. It can be assumed that in a more homogeneous group of subjects (by sex, age, height, body weight, physical fitness, etc.), the scatter would be smaller. Selecting homogeneous groups is a potential direction for future research in this field of respiratory acoustics. Furthermore, the search for individual regression dependencies is a promising avenue.

It can be assumed that the influence of individual differences on the relationship under study becomes smoother when transitioning from individual data to averaged values. Therefore, a regression analysis was performed on the averaged values of RS power and pulmonary ventilation parameters at each load stage. However, when considering the entire range of achieved pulmonary ventilation values up to 100 L/min, the coefficient of determination is only 0.68. This is likely due to the manifestation of a non-linear relationship between

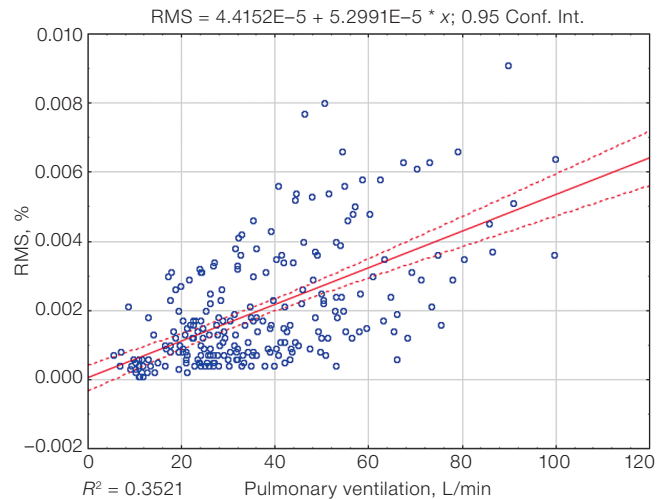


Figure prepared by the authors based on their own data

Fig. 3. Relationship between individual values of mean lung sound power and pulmonary ventilation parameters

Note: RMS — root mean square value of the lung sound amplitude.

RS power and pulmonary ventilation magnitude, as well as individual differences across the larger range of pulmonary ventilation data. Consequently, the search for a linear regression dependence should limit the range of pulmonary ventilation (to 60 L/min) (Fig. 4). In this case, the coefficient of determination R^2 increased significantly to 0.93. Beyond this point, the relationship deviated from linearity, and data scatter increased substantially, leading to a decrease in R^2 . In a similar manner, the correlation coefficient r_s decreased. This may be caused by the aforementioned non-linear phenomena and individual differences.

The practical significance of the obtained regression dependence lies in the possibility of assessing

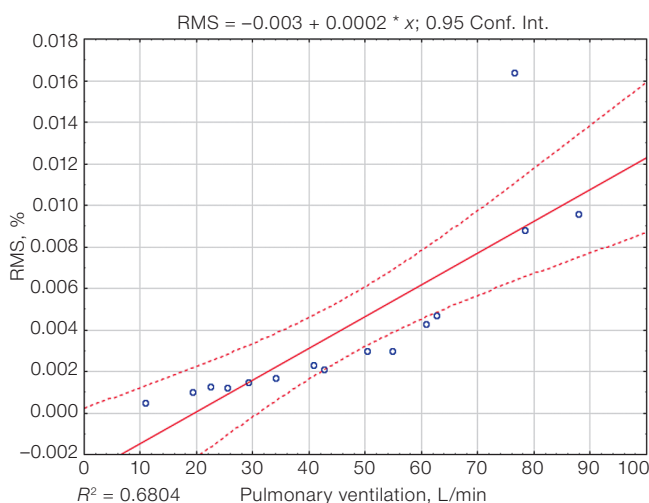
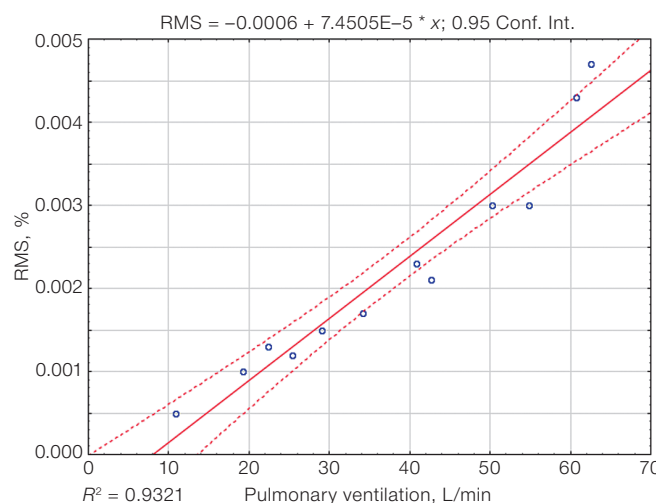


Figure prepared by the authors based on their own data

Fig. 4. Dependence of lung sound power on pulmonary ventilation magnitude based on average parameter values

Note: RMS — root mean square value of the lung sound amplitude.



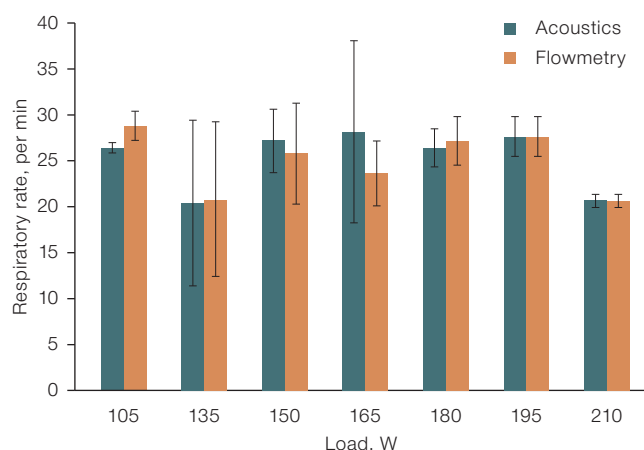


Figure prepared by the authors based on their own data

Fig. 5. Comparison of respiratory rate values obtained by the acoustic method with those by direct flowmetry at various levels of stepwise increasing physical load

the magnitude of pulmonary ventilation based on lung sounds.

To identify the degree of agreement between the respiratory rate (RR) values obtained by the acoustic method and those from direct flowmetry, a comparison was made between the acoustic assessment of RR and the flowmetric parameter (Fig. 5).

Figure 5 shows that at a load of 135 W, the RR according to acoustic data was minimal, while at a stepwise increase in physical load to 165 W, it was maximal. It appears that these extreme values are more influenced by individual variations in the

parameters. No significant differences were found between the RR values obtained by the two methods. The scatter in the values may be explained by differences in individual characteristics of the respiratory system.

CONCLUSIONS

1. A statistically significant, moderate positive correlation was established between the magnitude of pulmonary ventilation and the mean power of lung sounds ($r_s = 0.58$; $p < 0.001$).

2. For pulmonary ventilation values up to 60 L/min, the relationship between the mean power of tracheal sounds and pulmonary ventilation is linear. However, when this value is exceeded, additional non-linear processes are likely to contribute, altering the relationship. One such non-linear process could be the transition from laminar to turbulent airflow in the airways as the flow rate increases.

3. A comparison of the acoustic assessments of the respiratory system state with values based on direct flowmetry of respiratory flow showed satisfactory agreement. Consequently, the acoustic method can be considered an alternative to direct flowmetry in cases where the conventional approach is not applicable.

4. A potential direction for future research is the search for individual regression relationships and/or dependencies between the amplitude characteristic of the recorded lung sound signal and the magnitude of pulmonary ventilation in more homogeneous groups of subjects.

References

- Korenbaum VI, Pochekutova IA. *Acoustical-biomechanical interdependences in human forced expiratory production*. Vladivostok: Dal'nauka; 2006 (In Russ.). EDN: [VEJDKR](#)
- Massaroni C, Nicolò A, Lo Presti D, Sacchetti M, Silvestri S, Schena E. Contact-Based Methods for Measuring Respiratory Rate. *Sensors*. 2019;19(4):908. <https://doi.org/10.3390/s19040908>
- Rao A, Huynh E, Royston T, Kornblith A, Roy S. Acoustic Methods for Pulmonary Diagnosis. *IEEE Reviews in Biomedical Engineering*. 2019;12:221–39. <https://doi.org/10.1109/RBME.2018.2874353>
- Arts L, Lim EHT, van de Ven PM, Heunks L, Tuinman PR. The diagnostic accuracy of lung auscultation in adult patients with acute pulmonary pathologies: a meta-analysis. *Scientific Reports*. 2020;10:7347. <https://doi.org/10.1038/s41598-020-64405-6>
- Landry V, Matschek J, Pang R, Munipalle M, Tan K, Boruff J, et al. Audio-based digital biomarkers in diagnosing and managing respiratory diseases: a systematic review and bibliometric analysis. *European Respiratory Review*. 2025;34(176):240246. <https://doi.org/10.1183/16000617.0246-2024>
- Baranov VM. *Human gas and energy exchange in space flight and model research*. Moscow: Nauka; 1993 (In Russ.).
- Prisk GK. Microgravity and the respiratory system. *European Respiratory Journal*. 2014;43(5):1459–71. <https://doi.org/10.1183/09031936.00001414>
- Smith MB, Chen H, Oliver BGG. The Lungs in Space: A Review of Current Knowledge and Methodologies. *Cells*. 2024;13:1154. <https://doi.org/10.3390/cells13131154>
- Fomina EV, Uskov KV. Effectiveness of different training programs for physical performance maintenance in the condition of low motor activity. *Aerospace and Environmental Medicine*. 2016;50(5):47–55 (In Russ.). EDN: [WTNLFJ](#)
- Korenbaum VI, Pochekutova IA, Kostiv AE, Malaeva VV, Safronova MA, Kabantsova OI, et al. Human forced expiratory noise. Origin, apparatus and possible diagnostic applications. *Journal of the Acoustical Society of America*. 2020;148(6):3385–91. <https://doi.org/10.1121/10.0002705>
- Astafyeva SN, Zaripov RN, Mikhailovskaya AN, Dyachenko AI. Study of the ventilation function of the human lungs with the methods of respiratory acoustics during 5-day female dry immersion. *Aerospace and Environmental Medicine*. 2024;58(5):32–8 (In Russ.). <https://doi.org/10.21687/0233-528X-2024-58-5-32-38>
- Kostiv AE, Korenbaum VI. New possibilities for diver's monitoring during underwater diving using noises associated with

- natural respiration. *Ulyanovsk Medico-Biological Journal*. 2019;3:89–97 (In Russ.).
<https://doi.org/10.34014/2227-1848-2019-3-89-97>
13. Nekoutabar R, Ghaheiri FS, Jalilvand H. The Effect of Root-Mean-Square and Loudness-Based Calibration Approach on the Acceptable Noise Level. *Auditory and Vestibular Research*. 2024;33(4):361–70.
<https://doi.org/10.18502/avr.v33i4.16654>
 14. Khoshouei M, Bagherpour R, Jalalian M, Yari M. Investigating the acoustic signs of different rock types based on the values of acoustic signal RMS. *Rudarsko-geološko-naftni zbornik*. 2020;35(3):29–38.
<https://doi.org/10.17794/rgn.2020.3.3>
 15. Hubert P, Killick R, Chung A, Padovese LR. A Bayesian binary algorithm for root mean squared-based acoustic signal segmentation. *The Journal of the Acoustical Society of America*. 2019;146:1799–807.
<https://doi.org/10.1121/1.5126522>
 16. Nosovsky AM, Popova OV, Smirnov Yul. State-of-the art technologies of medical data statistical analysis and methods of graphic presentation. *Aerospace and Environmental Medicine*. 2023;57(5):149–54 (In Russ.).
<https://doi.org/10.21687/0233-528X-2023-57-5-149-154>
 17. Xie W, Hu Q, Zhang J, Zhang Q. EarSpiro: Earphone-based Spirometry for Lung Function Assessment. *Proceedings of the ACM on Interactive, Mobile, Wearable and Ubiquitous Technologies*. 2023;6(4):1–27.
<https://doi.org/10.1145/3569480>
 18. Banaszak EF, Kory RC, Snider GL. Phonopneumography. *American Review of Respiratory Disease*. 1973;107(3):449–55.
 19. Shykoff BE, Ploysongsang Y, Chang HK. Airflow and normal lung sounds. *American Review of Respiratory Disease*. 1988;137(4):872–6.
<https://doi.org/10.1164/ajrccm/137.4.872>
 20. Gavriely N, Cugell DW. Airflow effects on amplitude and spectral content of normal breath sounds. *Journal of Applied Physiology*. 1996;80(1):5–13.
<https://doi.org/10.1152/jappl.1996.80.1.5>
 21. Gavriely N, Palti Y, Alroy G. Spectral characteristics of normal breath sounds. *Journal of Applied Physiology*. 1981;50(2):307–14.
<https://doi.org/10.1152/jappl.1981.50.2.307>

Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. The greatest contributions are as follows: Svetlana N. Astafyeva — concept development, acoustic data collection, data analysis and interpretation, and draft manuscript preparation; Alexander I. Dyachenko — scientific supervision, manuscript review and editing; Irina A. Ruzhichko — research implementation (cycle ergometry); Anatoly E. Kostiv — provision of resources, approval of the final text.

AUTHORS

Svetlana N. Astafyeva

<https://orcid.org/0009-0004-7376-1713>
a10351@ya.ru

Alexander I. Dyachenko, Dr. Sci. (Tech.)

<https://orcid.org/0000-0002-5272-222X>
alexander-dyachenko@yandex.ru

Irina A. Ruzhichko

<https://orcid.org/0000-0003-3046-4757>
irina.ruzhichko@gmail.com

Anatoly E. Kostiv, Cand. Sci. (Tech.)

<https://orcid.org/0000-0002-7485-6499>
kostiv@poi.dvo.ru

<https://doi.org/10.47183/mes.2025-319>

EPIDEMIOLOGICAL ANALYSIS OF COSMONAUT MORTALITY: 1960–2023

Igor B. Ushakov¹, Andrei Yu. Bushmanov¹, Kristina V. Betts^{1✉}, Igor V. Bukhtiyarov², Yuri I. Voronkov³, Alexey P. Grishin⁴, Anatoliy F. Zhernavkov⁵¹ Burnazyan Federal Medical Biophysical Center, Moscow, Russia² Izmerov Research Institute of Occupational Health, Moscow, Russia³ Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia⁴ Gagarin Research and Test Cosmonaut Training Center, Zvyozdny Gorodok, Russia⁵ Association of Aerospace, Marine, Extreme and Environmental Medicine of Russia, Moscow, Russia

Introduction. Cohort analytical epidemiological studies of cosmonauts' health have a number of specific features. The estimates of long-term health outcomes in this occupational group can only be refined provided that the observation period of the Soviet and Russian cosmonaut cohort, originally established in 2013, be extended and regularly updated with emerging data.

Objective. Analysis of changes in the main mortality indicators of Soviet and Russian cosmonauts over the cohort observation period extended by five years until 2023.

Materials and methods. Using prospective observation studies over the historically formed cohort of male Soviet and Russian cosmonauts in 1960–2023, data update was implemented. Encoded and anonymized personal data of cosmonauts underwent processing and analysis. Using the standardized mortality ratio (SMR) with a 95% confidence interval (95% CI), mortality risk among cosmonauts was assessed. The total number of participants as of the cohort closure date (31.12.2023) was 270 cosmonauts, divided into two groups based on the presence of spaceflight (SF) experience. Group 1 comprised 127 cosmonauts (47.0%), who undertook at least one SF. Group 2 comprised 143 cosmonauts (53.0%) without SF experience (internal control). The male population of Russia served as the external control group. Cause-of-death analysis was conducted according to ICD-10. Additionally, information on the initial education of cosmonauts as a factor significantly influencing life trajectory was collected. Statistical processing was carried out using the MS and Stata 14 software packages.

Results. Extending the observation period contributed to an increased accuracy of death risk metrics by narrowing the 95% confidence intervals. The all-cause mortality risk among cosmonauts with SF experience was found to be reliably lower compared to both the male population of Russia (SMR = 0.37; 95% CI 0.27–0.50) and cosmonauts without SF experience (SMR = 0.73; 95% CI 0.53–0.98). No statistically significant decrease in cancer-related death risk was observed among cosmonauts with SF experience compared to the general population (SMR = 0.62, 95% CI 0.32–1.09). A correlation was found between the initial education of cosmonauts and mean age at death. Thus, the mean age of death for cosmonauts with military specialization and SF experience was 68.1 years, compared to 60.3 years ($p = 0.015$) for those without SF experience.

Conclusions. Oncological vigilance in cosmonauts with SF experience is generally higher than in both control groups, despite their unequivocally better health status. Further refinement of the data obtained can only be achieved by extending the observation period, considering the career-long duration of space stay in relation to the health status of cosmonauts. Individual-related factors affecting the health of cosmonauts without SF experience were identified. The link between professional education and the mean age at death underscores the need for developing individual preventive measures for this group and longer post-career health monitoring.

Keywords: cosmonauts; space flight; mortality; cohort studies

For citation: Ushakov I.B., Bushmanov A.Yu., Betts K.V., Bukhtiyarov I.V., Voronkov Yu.I., Grishin A.P., Zhernavkov A.F. Epidemiological analysis of cosmonaut mortality: 1960–2023. *Extreme Medicine*. 2026;28(1):102–112. <https://doi.org/10.47183/mes.2025-319>

Funding: the study was carried out without sponsorship.

Potential conflict of interest: Igor B. Ushakov, Andrei Yu. Bushmanov are members of the Editorial Board of *Extreme Medicine*. The other authors declare no conflict of interest.

✉ Kristina V. Betts c.betts@yandex.ru

Received: 16 June 2024 **Revised:** 1 Oct. 2025 **Accepted:** 15 Sept. 2025 **Online first:** 30 Oct. 2025

УДК 613.62:613.648.4:629.786.2

ЭПИДЕМИОЛОГИЧЕСКИЙ АНАЛИЗ СМЕРТНОСТИ КОСМОНАВТОВ: 1960–2023

И.Б. Ушаков¹, А.Ю. Бушманов¹, К.В. Беттс^{1✉}, И.В. Бухтияров², Ю.И. Воронков³, А.П. Гришин⁴, А.Ф. Жернавков⁵¹ Государственный научный центр Российской Федерации — Федеральный медицинский биофизический центр им. А.И. Бурназяна, Москва, Россия² Научно-исследовательский институт медицины труда им. академика Н.Ф. Измерова, Москва, Россия³ Институт медико-биологических проблем Российской академии наук, Москва, Россия⁴ Научно-исследовательский испытательный центр подготовки космонавтов им. Ю.А. Гагарина, Звездный Городок, Россия⁵ Ассоциация авиационно-космической, морской, экстремальной и экологической медицины России, Москва, Россия

Введение. Когортные аналитические эпидемиологические исследования состояния здоровья космонавтов имеют ряд особенностей, связанных со спецификой изучаемой профессиональной группы. Уточнение отдаленных показателей здоровья можно ожидать только при условии расширения периода наблюдения за когортой космонавтов СССР и России, впервые сформированной в 2013 г., с систематическим обновлением расчетных данных.

Цель. Анализ изменений основных показателей смертности отечественных космонавтов при 5-летнем расширении периода наблюдения за когортой до 2023 г.

© I.B. Ushakov, A.Yu. Bushmanov, K.V. Betts, I.V. Bukhtiyarov, Yu.I. Voronkov, A.P. Grishin, A.F. Zhernavkov, 2025

Материалы и методы. С применением проспективного наблюдения за исторически сформированной когортой космонавтов СССР и России мужского пола за период с 1960 по 2023 г. выполнено обновление данных, с помощью показателя стандартизованного относительного риска (COP) с 95% доверительным интервалом (95% ДИ) оценен риск смерти космонавтов. Проведены обработка и анализ закодированных и обезличенных персональных данных космонавтов. Общее количество участников на дату закрытия когорты (31.12.2023) составило 270 космонавтов, разделенных в зависимости от наличия опыта космического полета (КП) на две группы: группа 1 — 127 человек (47,0%), космонавты, совершившие хотя бы один КП; группа 2 — 143 человека (53,0%), космонавты, не имеющие опыта КП («внутренний контроль»). Мужское население России принято за «внешнюю» контрольную группу. Анализ причин смерти проведен по укрупненным классам болезней в соответствии с МКБ-10. Дополнительно собрана информация об исходном образовании космонавтов как фактора, значительно влияющего на траекторию жизни. Статистическая обработка произведена с использованием пакета программного обеспечения Microsoft Office и программного обеспечения Stata 14.

Результаты. Увеличение периода наблюдения способствовало повышению точности показателей риска смерти космонавтов за счет сужения 95% ДИ. Риск смерти космонавтов, совершивших космический полет (КП), от всех причин смерти в совокупности достоверно ниже при сравнении как с мужским населением России (COP = 0,37; 95% ДИ 0,27–0,50), так и с космонавтами без опыта КП (COP = 0,73; 95% ДИ 0,53–0,98). Получено отсутствие достоверного снижения риска смерти космонавтов, имеющих опыт КП, от злокачественных новообразований при сравнении с населением (COP = 0,62, 95% ДИ 0,32–1,09). Показано наличие связи между исходным образованием космонавтов и средним возрастом смерти: средний возраст смерти космонавтов — военных специалистов, имеющих опыт КП, составил 68,1 года, не имеющих опыта КП — 60,3 года ($p = 0,015$).

Выводы. Для космонавтов, совершивших КП, сохраняется общая онкологическая настороженность, несмотря на однозначно лучшее состояние их здоровья при сравнении с обеими контрольными группами. Уточнение полученных данных может быть достигнуто только при расширении периода наблюдения, в том числе за счет увеличения времени, проведенного в космосе за карьеру, в зависимости от жизненного статуса космонавтов. Обнаружено наличие внутренних факторов, влияющих на здоровье космонавтов без опыта КП: связь между профессиональным образованием и средним возрастом смерти говорит о необходимости разработки индивидуальных профилактических мероприятий для этой группы лиц, а также углубленного наблюдения за состоянием их здоровья после завершения карьеры.

Ключевые слова: космонавты; космический полет; смертность; когортные исследования

Для цитирования: Ушаков И.Б., Бушманов А.Ю., Бетц К.В., Бухтияров И.В., Воронков Ю.И., Гришин А.П., Жерनावков А.Ф. Эпидемиологический анализ смертности космонавтов: 1960–2023. *Медицина экстремальных ситуаций*. 2026;28(1):102–112. <https://doi.org/10.47183/mes.2025-319>

Финансирование: работа выполнена без спонсорской поддержки.

Потенциальный конфликт интересов: И.Б. Ушаков, А.Ю. Бушманов — члены редакционной коллегии журнала «Медицина экстремальных ситуаций». Остальные авторы заявляют об отсутствии конфликта интересов.

✉ Бетц Кристина Валерьевна c.betts@yandex.ru

Статья поступила: 16.06.2024 **После доработки:** 15.09.2025 **Принята к публикации:** 03.10.2025 **Online first:** 30.10.2025

INTRODUCTION

Cohort analytical epidemiological studies are generally applied for assessing causal relationships between exposure to negative factors and health outcomes in a specific risk group [1]. Cohort studies possess a number of undeniable strengths, including high ethical safety due to the absence of active intervention in the participants' lives and low susceptibility to systematic errors due to strict data collection protocols and the application of powerful statistical analysis methods. However, their implementation is associated with a significant drawback related to the necessity for a long-term observation of the studied group [2]. Depending on the research objective (e.g., morbidity or mortality assessment), the duration of observation for a risk population can range from several years to several decades, driven by the need to record the studied events in a sufficient number of participants to achieve statistical significance of the results.

Cohort studies of the health status of cosmonauts face additional challenges. First, the relatively small number of individuals under observation can, on one hand, reduce the statistical power of analysis, but, on the other, decrease the likelihood of losing cohort participants. Second, the unique health requirements,

multistage professional selection, lifestyle specifics, and socioeconomic status — all these factors impose special demands on the selection of a representative control group. Third, such studies are specific in terms of analyzing exposure time to adverse occupational conditions. In other professional groups, exposure is typically equal to the length of service; however, for cosmonauts, it is the duration of space stay over their career, which is much shorter than their total service record. Further development of manned spaceflight implies a constant increase in the duration of space missions, which extends the necessary observation period for such cohorts in analytical epidemiological studies. Fourth, access to some data is restricted and there are difficulties in tracking life trajectories after the end of a cosmonaut's career.

Space flights (SF) place immense physiological and psychological demands on the human body, making assessment of their long-term consequences a critical aspect of further space exploration [3, 4]. In this connection, in 2013, a large-scale cohort analytical study was initiated to cover a 53-year observation period of the cohort of USSR and Russian cosmonauts (1960–2013) [5]. This work was the first attempt to analyze mortality among cosmonauts, both those who had flown to space and those who had not, becoming an important

step in understanding the long-term effects of the complex impact of SF factors. However, as argued above, a comprehensive analysis can only be achieved by ensuring the continuity of the observation period and regularly supplementing emerging data.

In 1992, a long-term health monitoring program for astronauts was launched in the USA, entitled “Lifetime Surveillance of Astronaut Health” (LSAH). This program involves regular surveillance of morbidity and mortality, regularly updating the results obtained. The most complete study of astronaut mortality, covering the 1959–2017 period, was published in 2018 [6]. In 2021, this research was supplemented with a special focus on mortality from malignant neoplasms [7]. The experience of long-term continuous monitoring of the astronaut cohort demonstrates the validity and applicability of this approach. Further, monitoring of the international cohort of astronauts from EU countries, Canada, China, and Japan has been initiated [8], the results of which can be evaluated in the future.

Given the rarity of the studied event (mortality), updating the data every five years can be considered advisable. The previous update of the formed cohort of USSR and Russian cosmonauts was performed in 2018 (covering a 58-year period). In this work, we aim to analyze changes in the main mortality indicators in the cohort of USSR and Russian cosmonauts with an extended observation period by five years, until 2023 (in total, covering a 63-year period).

MATERIALS AND METHODS

The research was conducted by extending the observation period of the historical (retrospective) cohort of male cosmonauts by five years and its updating with new data. This cohort was first formed in 2013 and updated in 2018 [5, 9]. The observation period was extended until 31.12.2023 and updated with new death cases, person-years of observation, and data on new cosmonauts selected into the corps in 2018 and 2020. The total observation period was 63 years (1960–2023).

The sources of information on USSR and Russian cosmonauts were archival materials from the Medical Directorate of the Gagarin Research and Test Cosmonaut Training Center, archival materials from the Institute of Biomedical Problems of the Russian Academy of Sciences, and the open digital database “Roscosmos.”¹ The primary criterion for including cosmonauts in the study was the completion of basic space training.

The cosmonauts’ personal data were coded and anonymized. The total number of participants at the cohort closure date (31.12.2023) was 270 cosmonauts, who were divided into two groups based on space flight (SF) experience:

- Group 1 — 127 individuals (47.0%), cosmonauts who have completed at least one SF;

- Group 2 — 143 individuals (53.0%), cosmonauts without SF experience (internal control).

The relative numerical stability of the second group should be noted, which is due to parallel changes in the compositions of the cosmonaut groups: the enrollment of new cosmonauts into the corps and their transition from the second to the first group after completing a SF.

The main characteristics of the cohort are presented in Table 1.

Each cosmonaut was observed from the date of enrollment as a trainee in the corps and followed either until the date of death (for deceased individuals) or until the cohort closure date (31.12.2023). For a small portion of cosmonauts (from Group 2), the vital status could not be determined (2.8%), which allowed the study to continue by excluding them from the analysis.

The analysis of causes of death was conducted according to the following broad classes of diseases in accordance with the International Classification of Diseases, 10th Revision (ICD-10):

- malignant neoplasms (MN, C00–C97);
- diseases of the circulatory system (DCS, I00–I99);
- external causes, such as accidents during aviation and space flights, etc. (V01–Y98);
- other causes (not belonging to the specified classes, e.g., diseases of the endocrine system, diseases of the digestive organs, etc.);
- all causes combined.

The combination of all causes, excluding external ones, was taken as natural causes of death.

To identify additional factors that may have influenced cosmonaut mortality, information about their basic education was collected. Participants were assigned the following attributes:

- “Military specialist” — those who graduated from any military aviation school or college (for military pilots and navigators), as well as those who graduated from a military educational institution with an engineering specialty (for military engineers);
- “Engineer” — those who graduated from any civilian educational institution with one of the engineering specialties;
- “Other profession” — those who received other education, e.g., physician, journalist, biologist, etc.

The general characteristic of the cohort in terms of education is presented in Table 2.

The overwhelming majority of military specialists (pilots, navigators, and engineers) among cosmonauts in both groups is explained by the specifics of cosmonaut selection in the USSR. Then, selections were mainly conducted as closed departmental competitions, only occasionally supplemented by small thematic targeted recruitments (e.g., the selection from the USSR Academy of Sciences for filming a feature film aboard the *Mir* space station, the “Space is for Children” program, etc.). It was only in 2012 that

¹ Digital database of the state corporation for space activities “Roscosmos”. URL: <http://www.roscosmos.ru/>

Table 1. Vital status and age characteristics of the studied cosmonaut cohort, 1960–2023

Vital status	Group 1 <i>n</i> = 127		Group 2 <i>n</i> = 143		Total <i>n</i> = 270	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Living	82	64.6%	69	48.3%	151	55.9%
Deceased	45	35.4%	70	49.0%	115	42.6%
Unknown	0	0.0%	4	2.8%	4	1.5%
Total	127	100%	143	100%	270	100%
Mean age of cosmonauts, years						
Mean age of cosmonauts (years) at the time of	<i>M</i> ± <i>SD</i>		<i>M</i> ± <i>SD</i>		Statistical significance level, <i>p</i>	
Selection	31.2 ± 4.9		31.6 ± 5.9		0.544	
Living	63.9 ± 13.6		70.1 ± 16.6		0.007	
Deceased	67.7 ± 15.1		61.7 ± 15.3		0.021	

Table compiled by the authors based on their own data

Note: *n* — cosmonaut number; *p* — level of statistical significance; data are presented as mean value and standard deviation of the mean *M* ± *SD*.

Table 2. Initial education of cosmonauts

Initial education	Group 1		Group 2		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Military specialists, of which:	71	55.9%	86	61.9%	157	59.0%
Pilot	65	91.5%	63	73.3%	128	81.5%
Pilot-navigator	0	0.0%	4	4.7%	4	2.5%
Military engineer	6	8.5%	19	22.1%	25	15.9%
Civilian specialists, of which:	56	44.1%	53	38.1%	109	41.0%
Engineer	47	83.9%	34	64.2%	81	74.3%
Physician	6	10.7%	11	20.8%	17	15.6%
Civilian pilot	1	1.8%	1	1.9%	2	1.8%
Economist	1	1.8%	0	0.0%	1	0.9%
Biologist	1	1.8%	1	1.9%	2	1.8%
Journalist	0	0.0%	1	1.9%	1	0.9%
Physicist-Mathematician	0	0.0%	3	5.7%	3	2.8%
Actor	0	0.0%	1	1.9%	1	0.9%
Film/stage director	0	0.0%	1	1.9%	1	0.9%
Total	127	100%	139	100%	266	100%

Table compiled by the authors based on their own data

Note: *n* — cosmonaut number.

selections became open to a wider range of specialists and young people.

A comparative analysis of mortality among cosmonauts with SF experience was performed using internal and external control groups. The “internal” control group was formed from cosmonauts without SF experience, which helped minimize the influence of the “healthy worker” effect due to similar health characteristics [10]. The male population of Russia was taken as the “external” control group. The sources of information on causes of mortality in the male population of Russia in 1960–2023 were the Russian demographic database,² Rosstat data,³ the Demographic Yearbook of Russia,⁴ and the Russian Statistical Yearbook.⁵

Due to restrictions on the publication of data on mortality from external causes in the Russian population in effect since 2023, a separate comparative analysis of mortality for this category was not conducted.

The risk of death among cosmonauts was assessed based on the standardized mortality ratio (SMR), calculated using formula (1). The statistical significance of SMR was assessed using a 95% confidence interval (95% CI). Standardization was performed using the indirect method.

$$SMR = \frac{m^{cosm}}{\sum n_i^{cosm} \times R_i^{cont}}, \tag{1}$$

where m^{cosm} — total number of deceased in the cosmonaut cohort (group);
 n_i^{cosm} — number of person-years of observation for the i -th age subgroup in the cosmonaut cohort (group);
 $R_i^{cont} = m_i^{cont} / n_i^{cont}$ — estimate of absolute risk for the i -th age subgroup in the control group;

m_i^{cont}, n_i^{cont} — number of deaths and number of person-years of observation for the i -th age subgroup in the control group.

Statistical processing of the data obtained was carried out using the MS and Stata 14 software packages. Using the Shapiro–Wilk test to assess the normality of distribution and Student’s t -test, the mean values and their standard errors were calculated. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Structure of causes of death among cosmonauts with and without SF experience

As of the cohort closure date of 31.12.2023, the number of person-years of observation was 9119. Data on the vital status of the cosmonauts are presented in Table 1. The main causes of death for cosmonauts in Groups 1 and 2 are presented in Table 3.

In both cosmonaut groups, diseases of the circulatory system rank first in the structure of known causes of death (Group 1 — 53.3%; Group 2 — 22.9%). Among cosmonauts with SF experience, mortality from malignant neoplasms was recorded in 26.7% of cases, while mortality from external causes accounted for 13.3% of cases. In the group of cosmonauts without SF experience, mortality from external causes accounted for 18.6% of cases and ranked second in the mortality structure. Malignant neoplasms ranked third, being the cause of death in 15.7% of cases.

Our study cannot offer a complete picture of the cosmonaut mortality structure due to the large proportion of

Table 3. Causes of death among cosmonauts with and without SF experience during the observation period of 1960–2023

Causes of death (ICD-10)	Group 1		Group 2		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Diseases of the circulatory system (I00–I99)	24	53.3%	16	22.9%	40	34.8%
Malignant neoplasms (C00–C97)	12	26.7%	11	15.7%	23	20.0%
External causes (V01–Y98)	6	13.3%	13	18.6%	19	16.5%
Other causes (not belonging to the specified classes)	3	6.7%	1	1.4%	4	3.5%
Unknown	0	0.0%	29	41.4%	29	25.2%
All causes combined	45	100%	70	100%	115	100%

Table compiled by the authors based on their own data

Note: *n* — cosmonaut number.

² Russian Demographic Database of the Center for Demographic Research at the Russian Economic School. URL: <http://demogr.nes.ru/>
³ Federal State Statistics Service (Rosstat). URL: <https://rosstat.gov.ru/>
⁴ Demographic Yearbook of Russia 2023: Стат. сб. — Moscow: Rosstat; 2023.
⁵ Russian Statistical Yearbook 2024: Stat. collection — Moscow: Rosstat; 2024.

unknown causes of death among cosmonauts from the second group. For 29 individuals (41.4%) selected into the corps but without SF experience, data on the cause of death is lacking due to significant difficulties in tracking life trajectories after the end of their cosmonaut careers. For this reason, the calculation of cause-specific standardized mortality ratios for cosmonauts in Group 2 was not performed.

Mortality of cosmonauts with SF experience compared to the male population of Russia (external control)

Extending the observation period of the cosmonaut cohort from 58 to 63 years led to a narrowing of the confidence interval boundaries for the studied indicators, thus enabling more precise results to be obtained. Table 4 shows the SMR dynamics for death causes among cosmonauts who completed SF, considering all causes and specific disease categories, compared to the male population of Russia.

The mortality risks for cosmonauts from all indicated causes remain lower compared to the male population of the country. A slight increase in the risk of death from malignant neoplasms attracts attention, with the SMR rising from 0.60 (95% CI 0.29–1.12) in 2018 to SMR = 0.62 (95% CI 0.32–1.09). Although being minimal, this increase is of interest due to the narrowing of the 95% CI to a borderline value. The confidence interval is a range that contains the true value of a parameter with a probability of 95% [11]. In cases where the confidence interval includes the value of 1, the differences cannot be considered statistically significant. In earlier studies, the upper limit of the 95% CI for the SMR from malignant neoplasms significantly exceeded 1, evidently not allowing the interpretation of the reduced risk as reliable. In the present study, the upper limit of the 95% CI has narrowed to a value of 1.09, which raises additional questions for researchers.

The trend toward an increased risk of cosmonaut mortality from malignant neoplasms can be explained by several reasons. First, a number of researchers point out that mortality due to malignant neoplasms is less susceptible to the influence of the “healthy worker effect” (HWE) than other natural causes, due to the long latent period of development [12–14]. Moreover, the strength of the HWE itself decreases over time from the onset of employment: the HWE is most significant at a younger age at the beginning of a career. Along with an increase in the length of service and age, the influence of the HWE decreases, and the worker’s health status approaches that of the general population to which they belong [15]. Thus, for analyzing cosmonaut mortality from malignant neoplasms, the general population can be used as a comparison group, and the dynamics of this particular indicator will be of great interest in the future.

Second, Reynolds et al. [7] established increased risks for the development of certain types of cancer among astronauts (compared to the general US population), particularly skin melanoma. Although a direct link to astronauts’ working conditions was not established, the authors noted increased risks for both standardized incidence ratio (SIR 252; 95% CI 126–452) and mortality (SMR 508; 95% CI 105–1485) specifically due to skin melanoma. However, such results are characteristic of aircraft pilots, who constitute the majority among US astronauts (235 (69.5%) out of 338 individuals) and who are more exposed to ultraviolet radiation than to cosmic radiation. Among the cosmonauts in the studied cohort, the proportion of pilots (military and civilian) was also significant (52.0% among those who completed a SF; 46.0% among those who did not), but no deaths from skin melanoma were recorded in either group.

The aforementioned US study [7] also indicated a higher risk of incidence (but not mortality) among astronauts due to prostate cancer development (SIR 162; 95% CI 109–232), which the authors associate with earlier and more thorough screening that begins at age 40

Table 4. Standardized mortality ratios for cosmonauts with SF experience, compared to the male population of Russia, by cause of death and observation period

Causes of death	Observation period, years					
	1960–2018			1960–2023		
	SMR	95% CI		SMR	95% CI	
All causes of death	0.39	0.28	0.54	0.37	0.27	0.50
Malignant neoplasms	0.60	0.29	1.12	0.62	0.32	1.09
Diseases of the circulatory system	0.42	0.26	0.66	0.45	0.29	0.67

Table compiled by the authors based on their own data

Note: SMR — standardized mortality ratio; 95% CI — 95% confidence interval.

for this professional group, compared to age 50 for the general population. In Russia, according to current clinical guidelines,⁶ men over 50 are also considered at risk for prostate cancer development, requiring screening diagnostic methods (determination of prostate-specific antigen (PSA) in the blood). PSA testing is included in the annual medical examination program for cosmonauts — members of ISS crews, also starting from age 40, similar to astronauts. It is worth noting that during our study, two cases of death due to prostate cancer were recorded among cosmonauts with SF experience; however, the results seem too premature to discuss causal relationships. In the future, while continuing to monitor the established cosmonaut cohort, it is necessary to maintain oncological vigilance, especially considering the increase in the duration of space stay among deceased cosmonauts.

The total duration of completed SF as of 31.12.2023, was 31,207 days (of which 4048 days (12.9%) were accounted for by now-deceased cosmonauts). The average time spent in SF conditions per career for one living cosmonaut was 331.8 days, while for a now-deceased cosmonaut it was 76.5 days ($p < 0.01$). During the course of the study, differences in causes of death

depending on the average duration of SF have not been established, which is an object for future research.

In our study, a comparative analysis of cosmonaut mortality from external causes was not conducted, which is due to the absence of data on mortality from this class of causes in the Russian population. In a previously conducted study [9] covering the period of 1960–2018, the risk of death from external causes for cosmonauts with SF experience was 0.39 (95% CI 0.14–0.85) compared to the male population of Russia. No new cases of cosmonaut death from external causes were registered upon extending the observation period, which eliminates the theoretical possibility of an increased risk.

Mortality of cosmonauts with SF experience compared to cosmonauts without such experience (internal control)

The study of trends in the SMR among cosmonauts with SF experience compared to the internal control — cosmonauts without SF experience — with an increase in observation duration deserves separate attention (Table 5).

Table 5. Standardized mortality ratios for all causes combined for cosmonauts with SF experience, compared to cosmonauts without SF experience, by observation period

Observation period, years	SMR	95% CI	
1960–2018	0.66	0.46	0.91
1960–2019	0.70	0.50	0.95
1960–2020	0.68	0.49	0.93
1960–2021	0.68	0.49	0.92
1960–2022	0.71	0.51	0.95
1960–2023	0.73	0.53	0.98

Table compiled by the authors based on their own data
Note: SMR — standardized mortality ratio; 95% CI — 95% confidence interval.

Table 6. Mean age at death of cosmonauts from natural causes, depending on SF experience and observation period

Observation period, years	Mean age at death (natural causes), years		Statistical significance level, p
	With space flight experience	Without space flight experience	
1960–2018	68.8	63.1	<0.05
1960–2023	71.7	65.8	<0.05

Table compiled by the authors based on their own data
⁶ Clinical guidelines "Prostate Cancer"; 2021. URL: https://cr.minzdrav.gov.ru/preview-cr/12_3

A gradual increase in the SMR from all causes is observed for cosmonauts with SF experience compared to cosmonauts without SF experience, although remaining statistically significant across all periods. In 2018, the risk of death for cosmonauts who had completed a SF was significantly lower by 34% (SMR = 0.66; 95% CI 0.46–0.91). The five-year extension of the observation period somewhat smoothed out the difference in the risk of death — to 27% (SMR = 0.73; 95% CI 0.53–0.98). A narrowing of the CI boundaries was also noted, with the upper CI boundary gradually approaching the threshold value (1), beyond which the differences lose statistical significance.

The difference in the mean age at death from natural causes between the two cosmonaut groups also remained significant (Table 6).

With the extension of the cohort observation period, an increase in the mean age at death was recorded in both groups: among cosmonauts with SF experience by 2.9 years (to 71.7 years) and by 2.7 years (to 65.8 years) in the group of cosmonauts without SF experience. It is important to note that the trend toward earlier mortality among cosmonauts who did not complete a SF persisted: their mean age at death was almost six years less compared to cosmonauts who had flown to space ($p < 0.05$). The calculated risk and mean age at death indicate a worse health status of those cosmonauts who were selected into the corps but not into spacecraft crews.

Earlier works present additional examples to illustrate this fact. For instance, a separate study [16] was dedicated to the long-term (maximum 30 years) medical monitoring of 36 cosmonauts who had completed SF and 65 cosmonauts without SF experience. According to the authors, their long-term morbidity was more associated with age-related changes, while functional disorders of the cardiovascular system and hypertensive disease were more frequently recorded among cosmonauts without SF experience. In the study [17], survival functions were calculated for 30-year-old cosmonauts who had completed SF in the 1960s and cosmonauts without SF experience (34 and 59 individuals, respectively). The survival curve for cosmonauts who had not

completed a SF showed a clear increase in mortality in the age range of 55–60 years, subsequently falling below the analogous survival curve for cosmonauts with SF experience.

Undoubtedly, the anticipation of being assigned to a prime crew is associated with significant psychoemotional stress, which only intensifies over time in the absence of professional fulfillment. A study based on the analysis of 91 biographies of Russian cosmonauts and 14 biographies of US astronauts established that the anticipation of a SF and the risk of being discharged from the corps are the most negative stress factors affecting cosmonauts' health [18]. A SF itself can be viewed as a source of distress (the negative component of stress), but also as a source of eustress — the positive component of stress that mobilizes the body's protective functions and compensates for the impact of negative SF factors. Cosmonauts who have not completed a SF find themselves in a worse position compared to cosmonauts with SF experience due to the absence of eustress. In the aforementioned study, the average lifespan of non-flying cosmonauts was shorter than that of flying cosmonauts (59.3 and 65.9 years, respectively).

Chronic stress, including that associated with professional activity, negatively affects the body's homeostasis, with its link to morbidity and mortality having been described by many authors [19, 20]. However, the direction of the causal relationship can be interpreted in two ways: prolonged denial of flight clearance can cause a deterioration in health status, and conversely, identified poorer health indicators can be the reason for denial of SF clearance.

Overall, the health of cosmonauts, even those who have not completed a SF, is significantly better compared to the male population of Russia. Thus, the SMR, calculated considering all causes of death for this group over the 63-year observation period, was 0.47 (95% CI 0.37–0.59).

The identified mortality indicators for cosmonauts without SF experience, which are worse compared to cosmonauts who have completed SF, indicate the existence of other factors and individual-level mechanisms affecting their health. In an attempt to search for such

Table 7. Mean age at death of cosmonauts with and without SF experience, by initial education

Initial education	With SF experience, years	Without SF experience, years	Statistical significance level, p
Engineers	67.5 ± 16.5	67.9 ± 15.7	0.519
Military specialists	68.1 ± 15.5	60.3 ± 15.3	0.015
Others	65.5 ± 10.2	59.8 ± 12.9	0.249

Table compiled by the authors based on their own data

Note: p — level of statistical significance; data are presented as mean value and standard deviation of the mean $M \pm SD$.

factors, we carried out an analysis of the mean age at death of cosmonauts depending on their initial education (Table 7). It is important to note that the mean age of enrollment in the cosmonaut corps showed no differences based on either education or SF experience, ranging 30.0–33.3 years for all cosmonauts.

In the structure of initial education, the overwhelming majority of cosmonauts were military specialists (55.9% in Group 1 and 61.9% in Group 2), while civilian engineers ranked the second (37.0% and 24.5%, respectively) (Table 2). When assessing the mean age at death of engineers and cosmonauts with other education in both groups, no statistically significant differences were established. However, a significant difference in the mean age at death was shown for military specialists: for cosmonauts who graduated from military educational institutions and did not complete a SF, the mean age at death was 60.3 years, which is eight years less compared to cosmonauts with the same education but with SF experience (68.1 years, $p = 0.015$).

Furthermore, when the analysis was limited to data on military pilots, who constitute the majority among military specialists (91.5% in Group 1; 73.3% in Group 2), the differences became even more substantial: the mean age at death for pilots with SF experience was 67.7 years, compared to 56.7 years ($p = 0.006$) for those without SF experience. No statistically significant differences in the mean age at death were obtained for military engineers. This observation might be explained by the methods and pathways for realizing professional potential, which differ significantly for civilian and military specialists [21]. For engineers, the unique experience gained during the recruitment process and time spent in the cosmonaut corps is a valuable symbolic capital, actively exploited later when changing their field of activity. For military specialists, unlike civilian ones, career strategy is aimed at achieving specific objectives, and a SF itself is an important goal on this path.

The training conditions for military and civilian specialists also differed: cosmonauts with a civilian profession were not required to be permanently stationed at the Gagarin Cosmonaut Training Center. In the absence of prospects for a SF, they could return to their primary jobs, which was impossible for cosmonauts whose military profession limited their professional mobility.

Regarding pilots, it is necessary to remember that people in this group (especially pilots of high-maneuverability aircrafts) should possess certain personality characteristics prior to selecting this profession [22]. This was convincingly demonstrated earlier in fundamental and applied experimental psychological studies [23]. Therefore, the personality type of a pilot is also a determining factor in the established pattern.

The group of cosmonauts selected into the corps but not cleared for SF deserves closer attention and

thorough study of their health status, including mortality, for a number of reasons. On the one hand, the very fact of successfully passing the professional selection process into the cosmonaut corps already confirms the high qualifications and good health status of this group of individuals, who face additional negative factors throughout their careers and while awaiting flight assignment. This necessitates the development of measures to prevent early mortality, including personalized medical and psychological assistance. On the other hand, comprehensive data on the health of cosmonauts who were selected but did not complete a SF is key to establishing the true long-term effects of spaceflights on the human body, since this professional group can serve as a unique “internal” control in analytical epidemiological studies of the health of cosmonauts who have completed SF. To date, among cosmonauts without SF experience, the proportion of individuals with unknown causes of death remains significant at 41.4% (Table 3), which prevents a comparative analysis of mortality by specific causes.

CONCLUSION

Regular five-year extensions of the observation period for the historical cohort of Soviet and Russian cosmonauts have established that the risk of death for cosmonauts with SF experience remains undeniably lower compared to the male population of Russia. However, a general oncological vigilance in this group continues to exist, confirmed both by the lack of reliability in the reduced risk of death from malignant neoplasms and by the results obtained by US researchers. Currently, the time spent in space by deceased cosmonauts over their careers remains relatively small compared to the total duration of completed SF, which prevents straightforward conclusions about the connection between cosmonauts' working conditions and such a long-term health indicator as mortality. Based on the observed changes in the calculated values obtained under a longer observation period, it can be unequivocally stated that further research in this area is necessary. Future directions should focus on the long-term consequences of the entire set of factors affecting cosmonauts both on Earth (e.g., medical radiation examinations) and in space (in particular, the duration of extravehicular activity). Solving these tasks is only possible by extending the observation period of the established cohort with regular updates of the calculated data.

Cosmonauts who have not completed a SF also possess better health compared to the general population. However, in this group, the role of other, individual-level factors reflected in mortality has been demonstrated. Thus, the established connection between initial education and the mean age at death indicates the necessity for in-depth monitoring of the health status of this cosmonaut group after leaving the corps. This information

can serve as the basis for developing therapeutic and preventive measures, including timely psychological support based on an individual, personalized approach.

Furthermore, elucidation of the causes of death among cosmonauts without SF experience can contribute to revealing other mechanisms affecting their health.

References

- Grimes DA, Schulz KF. Cohort studies: marching towards outcomes. *Lancet*. 2002;359(9303):341–5. [https://doi.org/10.1016/s0140-6736\(02\)07500-1](https://doi.org/10.1016/s0140-6736(02)07500-1)
- Kholmatova KK, Kharkova OA, Gribovski AM. Cohort studies in medicine and public health. *Human Ecology*. 2016;4:56–64 (In Russ.). <https://doi.org/10.33396/1728-0869-2016-4-56-64>
- Lebedeva MA, Medvedeva YuS, Baranov MV, Arutyunyan AV, Zolotov NN, Karganov NN. Delayed effects of simulated space flight factors. *Pathogenesis*. 2021;19(1):37–49 (In Russ.). <https://doi.org/10.25557/2310-0435.2021.01.37-49>
- Grigoriev AI, Egorov AD, Potapov AN. Some Medical Problems of the Manned Martian Expedition. *Aerospace and Environmental Medicine*. 2000;34(3):6–12 (In Russ.).
- Ushakov IB, Voronkov Yul, Bukhtiyarov IV, Tikhonova GI, Gorchakova TYu. Retrospective health assessment of cosmonauts after participation in space flights. *Aerospace and Environmental Medicine*. 2016; 50(2):14–20 (In Russ.). <https://doi.org/10.21687/0233-528X-2016-50-2-14-20>
- Reynolds RJ, Day SM. *The mortality of space explorers. Into Space — A Journey of How Humans Adapt and Live in Microgravity*. Ed. by Russomano T, Rehnberg L. London: IntechOpen. 2018:253–85. <https://doi.org/10.5772/intechopen.73603>
- Reynolds R, Little MP, Day S, Charvat J, Blattnig S, Huff J, et al. Cancer incidence and mortality in the USA Astronaut Corps, 1959–2017. *Occupational and Environmental Medicine*. 2021;78(12):869–75. <https://doi.org/10.1136/oemed-2020-107143>
- Reynolds RJ, Day SM. Mortality Among International Astronauts. *Aerospace Medicine and Human Perform*. 2019;90(7):647–51. <https://doi.org/10.3357/amhp.5369.2019>
- Betts KV, Fateev IV. Mortality of Russian cosmonauts, 1960–2018. *Russian Journal of Occupational Health and Industrial Ecology*. 2020;60(11):730–3 (In Russ.). <https://doi.org/10.31089/1026-9428-2020-60-11-730-733>
- Chowdhury R, Shah D, Payal AR. Healthy Worker Effect Phenomenon: Revisited with Emphasis on Statistical Methods — A Review. *Indian Journal of Occupational and Environmental Medicine*. 2017;21(1):2–8. https://doi.org/10.4103/ijoom.ijoom_53_16
- Andreev EM, Zhdanov DA, Yasilionis D. Confidence estimation of demographic coefficients on the example of mortality rates. *Demographics Review*. 2015;2(1):24–55 (In Russ.). EDN: [VOFMJX](#)
- Kojiro K. The Healthy Worker Effect in a Long-term Follow-up Population. *Japanese Journal of Cancer Clinics*. 1999;45(12):1307–10.
- Fornalski KW, Dobrzyński L. The healthy worker effect and nuclear industry workers. *Dose-Response: An International Journal*. 2010;8(2):125–47. <https://doi.org/10.2203/dose-response.09-019.fornalski>
- Monson RR. Observations on the healthy worker effect. *Journal of Occupational and Environmental Medicine*. 1986;28(6):425–33. <https://doi.org/10.1097/00043764-198606000-00009>
- Chowdhury R, Shah D, Payal AR. Healthy Worker Effect Phenomenon: Revisited with Emphasis on Statistical Methods — A Review. *Indian Journal of Occupational and Environmental Medicine*. 2017;21(1):2–8. https://doi.org/10.4103/ijoom.ijoom_53_16
- Voronkov Yul, Kuzmin MP, Krylova VYu, Krivitsina ZA, Smirnov OA, Tizul AY, et al. Results of long-term observation of the health of cosmonauts. *Aerospace and Environmental Medicine*. 2002;36(1):41–4 (In Russ.). EDN: [RVZHQD](#)
- Shafirkin AV. Life expectancy of cosmonauts of the USSR (Russia) and astronauts of the United States and health risks under the influence of various stressors on Earth and in space. *Aerospace and Environmental Medicine*. 2019;53(7):5–18 (In Russ.). <https://doi.org/10.21687/0233-528X-2019-53-7-5-18>
- Berezina TN, Mansurov EI. The influence of stressogenic factors on the life expectancy of cosmonauts. *Questions of Psychology*. 2015;3:73–83 (In Russ.). EDN: [VMGQVD](#)
- Vancheri F, Longo G, Vancheri E, Henein MY. Mental Stress and Cardiovascular Health — Part I. *Journal of Clinical Medicine*. 2022;11(12):33–53. <https://doi.org/10.3390/jcm11123353>
- Kivimäki M, Pentti J, Ferrie JE, Batty GD, Nyberg ST, Jokela M, et al. Work stress and risk of death in men and women with and without cardiometabolic disease: a multicohort study. *Lancet Diabetes and Endocrinology*. 2018;6(9):705–13. [https://doi.org/10.1016/s2213-8587\(18\)30140-2](https://doi.org/10.1016/s2213-8587(18)30140-2)
- Akulov OY. Professional mobility of a military specialist and factors of its formation. *Bulletin of the Voronezh State Technical University*. 2014;10(3–2):85–90 (In Russ.). EDN: [SGITDZ](#)
- Shcherbakova EA. Personal characteristics of a high-class military pilot. *Bulletin of Adyghe State University. Series 3: Pedagogy and Psychology*. 2011;2:214–8 (In Russ.). EDN: [OEDGUH](#)
- Beregovoy GT, Zavalova ND, Lomov BF, Ponomarenko VA. *Experimental psychological research in aviation and cosmonautics*. Moscow: Nauka; 1978 (In Russ.).

Authors' contributions. All authors confirm that they meet the ICMJE criteria for authorship. The greatest contribution is distributed as follows: Igor B. Ushakov — project supervision, creation of the work concept; Andrei Yu. Bushmanov — approval of the final manuscript text; Kristina V. Betts — study design, data collection and analysis, manuscript creation and editing; Igor V. Bukhtiyarov — development of the study methodology, leadership in research planning; Yuri I. Voronkov — data verification; Alexey P. Grishin — material collection; Anatoliy F. Zhernavkov — project administration.

AUTHORS

Igor B. Ushakov, Dr. Sci. (Med.)
<https://orcid.org/0000-0002-0270-8622>
iushakov@fmbcfmba.ru

Andrei Yu. Bushmanov, Dr. Sci. (Med.)
<https://orcid.org/0000-0003-1565-4560>
abushmanov@fmbcfmba.ru

Kristina V. Betts, Cand. Sci. (Med.)
<https://orcid.org/0000-0002-2765-2856>
c.betts@yandex.ru

Anatoliy F. Zhernavkov, Cand. Sci. (Med.)
anatoliy.zhernavkov@mail.ru

Igor V. Bukhtiyarov, Dr. Sci. (Med.)
<https://orcid.org/0000-0002-8317-2718>
bukhtiyarov@irioh.ru

Yuri I. Voronkov, Dr. Sci. (Med.)
<https://orcid.org/0009-0004-4344-5066>
voronkov@imbp.ru

Alexey P. Grishin
<https://orcid.org/0000-0003-1429-1358>
a.grishin@gctc.ru

<https://doi.org/10.47183/mes.2025-409>



REQUIREMENTS FOR MEDICAL PERSONNEL PROVIDING MEDICAL CARE AT ONSITE INDUSTRIAL FACILITIES IN THE RUSSIAN ARCTIC CONTINENTAL SHELF

Oleg I. Orlov¹, Elena Yu. Mamonova^{1✉}, Michael A. Romanov², Maria Yu. Kalinina³

¹ Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia

² Gazprom Neft Shelf LLC, St. Petersburg, Russia

³ Burnazian Federal Medical Biophysical Center, Moscow, Russia

Introduction. The development of the Arctic zone of the Russian Federation implies the operation of offshore structures under extreme climatic conditions, high industrial hazards, and remoteness from onshore infrastructures. In such a context, provision of efficient medical care becomes of strategic importance. However, personnel training for health posts on offshore structures remains insufficient. In addition, there is no professional standard for a “shipboard medical specialist,” with the existing qualification requirements covering only a portion of all necessary competencies.

Objective. To analyze and generalize the regulatory, organizational, and personnel requirements for medical staff at health posts of offshore structures on the Arctic Shelf, and to develop proposals for improving their training system.

Materials and methods. The study was conducted using an analytical retrospective design. The research base included the regulatory legal acts of the Russian Federation (2010–2025), orders of the Ministry of Health, the Ministry of Labor, and the FMBA of Russia, corporate regulations of two oil and gas companies, as well as medical evacuation statistics. Methods of analytical review and content analysis were applied.

Results. Staff shortages and the lack of specialized training were established as the limiting factors in the sustainable functioning of the entire system. The necessity of a multi-level training model was substantiated, including mandatory qualification requirements, regular simulation trainings, specialized courses and introductory courses for physicians, as well as the requirement for certificates permitting physicians to work.

Conclusions. Regular additional training and the formation of a unified registry of physicians who have completed the full training program are key conditions for ensuring the medical safety of remote facilities in the Arctic zone of the Russian Federation and for developing the personnel potential.

Keywords: Arctic shelf; offshore structures; health post physician; qualification requirements; professional standards; additional training; simulation training

For citation: Orlov O.I., Mamonova E.Yu., Romanov M.A., Kalinina M.Yu. Requirements for medical personnel providing medical care at onsite industrial facilities in the Russian Arctic continental shelf. *Extreme Medicine*. 2026;28(1):113–122. <https://doi.org/10.47183/mes.2025-409>

Funding: the work was performed without sponsorship support.

Compliance with ethical principles: the study did not use personal data and did not involve interventions with patients. The analysis was based exclusively on the current legislation of the Russian Federation, regulatory legal acts of Federal executive bodies, as well as on the generalization of corporate regulations and practical experience in organizing medical support for offshore oil and gas platforms. In this regard, approval from an ethics committee was not required.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Elena Yu. Mamonova elenamamonova@mail.ru

Received: 2 Oct. 2025 **Revised:** 24 Nov. 2025 **Accepted:** 28 Nov. 2025 **Online first:** 30 Dec. 2025

УДК 616-01/-099

ТРЕБОВАНИЯ К МЕДИЦИНСКОМУ ПЕРСОНАЛУ, ОСУЩЕСТВЛЯЮЩЕМУ МЕДИЦИНСКУЮ ПОМОЩЬ НА СТАЦИОНАРНЫХ ПРОИЗВОДСТВЕННЫХ ОБЪЕКТАХ В РАЙОНЕ АРКТИЧЕСКОГО ШЕЛЬФА РОССИИ

О.И. Орлов¹, Е.Ю. Мамонова^{1✉}, М.А. Романов², М.Ю. Калинина³

¹ Институт медико-биологических проблем РАН, Москва, Россия

² ООО «Газпром нефть шельф»

³ Государственный научный центр Российской Федерации — Федеральный медицинский биофизический центр им. А.И. Бурназяна, Москва, Россия

Введение. Освоение Арктического шельфа Российской Федерации сопровождается эксплуатацией морских сооружений в условиях экстремального климата, высокой производственной опасности и удаленности от береговой инфраструктуры. В этих условиях обеспечение медицинской помощи приобретает стратегическое значение, однако подготовка кадров для здравпунктов морских сооружений остается недостаточной: отсутствует профессиональный стандарт «судовой медицинский работник», а действующие квалификационные требования охватывают лишь часть необходимых компетенций.

Цель. Провести анализ и систематизацию нормативных, организационных и кадровых требований к медицинскому персоналу здравпунктов морских сооружений Арктического шельфа и разработать предложения по совершенствованию системы их подготовки.

Материалы и методы. Исследование выполнено в аналитическом ретроспективном дизайне. В качестве базы использованы нормативно-правовые акты РФ (2010–2025 гг.), приказы Минздрава, Минтруда и ФМБА России, корпоративные регламенты двух

© O.I. Orlov, E.Y. Mamonova, M.A. Romanov, M.Y. Kalinina, 2025

нефтегазовых компаний, а также статистика медицинских эвакуаций. Применялись методы аналитического обзора и контент-анализа.

Результаты. Установлено, что кадровый дефицит и отсутствие специализированной подготовки ограничивают возможности устойчивого функционирования системы. Показана необходимость многоуровневой модели обучения, включающей обязательные квалификационные требования, регулярные симуляционные тренинги, специализированные курсы и вводный курс для врачей, а также наличие сертификатов, допускающих врачей к работе.

Выводы. Регулярная дополнительная подготовка и формирование единого реестра врачей, прошедших полную программу обучения, являются ключевыми условиями для обеспечения медицинской безопасности удаленных объектов Арктической зоны и развития кадрового потенциала отрасли.

Ключевые слова: Арктический шельф; морские сооружения; врач здравпункта; квалификационные требования; профессиональные стандарты; дополнительное обучение; симуляционные тренинги

Для цитирования: Орлов О.И., Мамонова Е.Ю., Романов М.А., Калинина М.Ю. Требования к медицинскому персоналу, осуществляющему медицинскую помощь на стационарных производственных объектах в районе Арктического шельфа России. *Медицина экстремальных ситуаций*. 2026;28(1):113–122. <https://doi.org/10.47183/mes.2025-409>

Финансирование: работа выполнялась без спонсорской поддержки.

Соответствие принципам этики: в исследовании не использовались персональные данные и не проводились вмешательства с участием пациентов. Анализ базировался исключительно на действующем законодательстве Российской Федерации, нормативно-правовых актах федеральных органов исполнительной власти, а также на обобщении корпоративных регламентов и практического опыта организации медицинского обеспечения на морских нефтегазовых платформах. В связи с этим одобрение этического комитета не требовалось.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Мамонова Елена Юрьевна elenamamonova@mail.ru

Статья поступила: 02.10.2025 **После доработки:** 24.11.2025 **Принята к публикации:** 28.11.2025 **Online first:** 30.12.2025

INTRODUCTION

The development of the Russian Arctic Zone is accompanied by active exploitation of the continental shelf and commissioning of stationary and floating offshore oil and gas facilities.¹ These facilities are operated under extreme climatic conditions and hazardous production factors, at the same time as being significant remote from onshore medical infrastructures. In such hubs of industrial activity, the issues of worker health protection are acquiring strategic importance. Provision of medical care in remote and hard-to-reach locations is impossible without specially trained medical personnel capable of functioning efficiently in autonomous conditions [1–7].

However, the system of personnel training for providing medical services in such conditions remains underdeveloped. The nomenclature of medical specialties is currently lacking a “maritime (shipboard) medicine” track. In addition, the professional standard for a “shipboard medical worker” requires revision. The existing qualification requirements are limited to the profiles of “general practice,” “therapy,” and “surgery,” which only partially correspond to the range of tasks faced by a health post physician serving at an offshore facility.

Medical personnel at offshore facilities are expected to effectively perform the functions of both a general practitioner, surgeon, anesthesiologist–intensivist, and public health physician, working under conditions of limited resources and the absence of a

possibility of rapid evacuation to shore. However, the current system of postgraduate education does not include specialized training that accounts for the specifics of working in Arctic maritime conditions. This creates a deficit of critically important competencies for providing advanced medical care, conducting sanitary-epidemiological control, and organizing evacuation in the setting of complete autonomy and extreme climatic factors [8].

Facing a shortage of specialists, employers are forced to independently develop personnel requirements and training programs. Major oil and gas companies are creating corporate standards that include mandatory courses in industrial and extreme medicine, sanitary-epidemiological control, and telemedicine interaction [9, 10]. While such initiatives partially compensate for legal and educational gaps, they are fragmented in nature and cannot replace an integrated approach to training physicians capable of safeguarding worker health in conditions of isolation and autonomy [11, 12].

In this study, we set out to analyze and generalize the regulatory, organizational, and personnel requirements for medical staff operating health posts at offshore facilities of the Arctic Shelf, with the subsequent development of proposals for establishing a unified physician training system. To that end, the following research objectives are set:

- to analyze the current regulatory framework of the Russian Federation governing the activities of

¹ GOST R 55311-2024. National Standard of the Russian Federation. “Oil and Gas Industry. Offshore Oil and Gas Production Facilities. Terms and Definitions” (approved and enacted by Order of Rosstandart No. 831-st dated 18.06.2024). URL: https://meganorm.ru/mega_doc/norm_update_05072025/gost-r_gosudarstvennyi-standart/0/gost_r_55311-2024_natsionalnyy_standart_rossiyskoy.html?ysclid=mhwzq877jw104846731

medical professionals working at offshore facilities of the Arctic Shelf in order to identify key gaps and contradictions;

- to generalize the organizational and personnel requirements for physicians of health posts at offshore facilities, including their qualifications, professional skills, work experience, and specific training for operating in autonomous and high-risk conditions;
- to develop proposals for establishing a unified system of basic training and continuing professional education for physicians working at onsite offshore facilities of the Arctic Shelf, taking into account remoteness, extreme climatic factors, and the necessity for interdisciplinary competencies.

MATERIALS AND METHODS

The search for scientific literature was conducted via electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Scopus, Google Scholar) languages. In addition, regulatory documents from the ConsultantPlus legal reference system and official governmental websites were analyzed.

The study employed an analytical research method, which included the examination and comparison of regulatory legal acts of the Russian Federation governing the organization of medical support on marine vessels and facilities of the Arctic Shelf (Federal Laws, Orders of the Ministry of Health of Russia, the Ministry of Labor of Russia, the Ministry of Transport of Russia, the FMBA of Russia, etc.), current orders and draft regulatory documents defining qualification requirements for medical specialists, the procedure for licensing medical activities, as well as regulations governing the operation of health posts.

The research involved an analysis of regulatory and methodological documents published in 2010–2025, draft professional standards and methodological recommendations from the FMBA of Russia, as well as internal regulations and technical specifications from oil and gas sector companies. Additionally, statistical data on the provision of all types of medical care at offshore structures was examined. Particular attention was paid to materials concerning the medical evacuation of workers to onshore healthcare facilities, with an assessment of the frequency, timelines, and outcomes of evacuation operations.

RESULTS AND DISCUSSION

Identified issues

The key challenge in organizing medical care at health posts on offshore structures, including marine oil platforms and support vessels, remains the provision of these facilities with qualified medical personnel capable of delivering the full range of medical services, including emergency² care, under conditions of remoteness from onshore infrastructure and limited resources [13].

A physician at such a facility must be prepared to perform functions that extend far beyond standard primary health care. These imply providing intensive therapy, maintaining vital functions until evacuation, conducting pre-shift medical examinations and, if necessary, testing for alcohol, narcotic, and other toxic substance use, implementing preventive measures, monitoring the sanitary-hygienic condition of production areas, common areas, food service, and water supply, and organizing medical triage in mass casualty incidents [14]. In the Arctic zone, where medical evacuation can take up to several days, such competencies become critically important for ensuring industrial safety and preserving workers' lives [15, 16].

In the Russian nomenclature of specialties approved by Order No. 700n³, the specialty "Maritime (Shipboard) Medicine" is absent, which hinders the formation of a comprehensive personnel training system. Nevertheless, Order No. 205n⁴ establishes the position of a "Physician on Shipboard", and Order No. 206n⁵ sets qualification requirements allowing specialists with certification in general medical practice, therapy, or surgery to hold this position. The General Medical Practice program provides the physician with interdisciplinary knowledge — from therapy and surgery to psychiatry, infectious diseases, and occupational pathology — which is crucial in conditions of limited access to specialized care.

At the Pirazlomnaya platform (the only onsite ice-resistant oil platform in the Russian Arctic), extensive experience has been accumulated in organizing the work of an anesthesiologist-resuscitator⁶ and obtaining the corresponding license to provide specialized medical care under the conditions of high industrial risks [17].

The absence of an approved professional standard for a "shipboard medical specialist" requires the employers to independently formulate additional qualification

² Federal Law of the Russian Federation No. 323-FZ "On the fundamentals of public health protection in the Russian Federation" dated 21.11.2011. URL: https://gb7omsk.gosuslugi.ru/netcat_files/8/9/323_FZ.pdf?ysclid=mhwzenpfgh546200203

³ Order of the Ministry of Health of the Russian Federation No. 700n "On the nomenclature of specialties for specialists with higher medical and pharmaceutical education" dated 07.10.2015. URL: <https://base.garant.ru/71250136/>

⁴ Order of the Ministry of Health of the Russian Federation No. 205n "On the approval of the nomenclature of positions for medical and pharmaceutical workers" dated 02.05.2023. URL: <https://www.garant.ru/products/ipo/prime/doc/406872398/?ysclid=mhbrddrmps968226694>

⁵ Order of the Ministry of Health of the Russian Federation No. 206n "On the approval of qualification requirements for medical and pharmaceutical workers with higher education" dated 02.05.2023. URL: <https://www.garant.ru/products/ipo/prime/doc/406869654/?ysclid=mg544wih8a549224078>

⁶ Order of the Ministry of Health of the Russian Federation No. 919n "On the approval of the procedure for providing medical care to the adult population in the specialty 'anesthesiology and resuscitation'" dated 15.11.2012. URL: <https://base.garant.ru/70301502/?ysclid=mhwz086pzs492303295>

requirements for physicians in the technical specifications for medical services. Consequently, alongside the mandatory requirements of a higher medical education in General Medicine, a valid certificate (accreditation), and confirmed practical experience of at least five years, there is a need for targeted additional training programs. These programs must ensure the physician's readiness for autonomous work at offshore facilities under conditions of remoteness and a high level of occupational risks [8, 13, 18].

Mandatory qualification requirements for medical personnel working at offshore health posts

Appointment to the position of a health post physician at an offshore facility requires the specialist to possess a higher medical education degree, confirmed by a valid license (or certificate of accreditation), and practical experience in the relevant specialty for at least five years after obtaining the specialist physician certificate. Priority is given to experience working in a remote health post of an industrial enterprise, which assumes the physician's readiness to perform professional duties in conditions of autonomy and limited resources [13].

The qualification level of medical personnel must strictly comply with the legislative requirements outlined in Order No. 541n⁷. Additionally, the physician is required to possess a specialist certificate or a state-accredited specialist credential, which must conform to the provisions of the professional standards for paramedics and physicians, as defined by Orders No. 470n⁸, No. 293n⁹, and No. 743n¹⁰. Modern practice emphasizes the importance of not only formal certification but also the alignment of specialists with professional and psychophysiological criteria. In particular, the physician must demonstrate stress resistance and be in a physical condition that allows them to perform procedures requiring rapid and precise motor coordination.

Particular attention is paid to the physician's readiness to provide emergency medical care. In accordance with Orders No. 911n¹¹, No. 543n¹², and No. 252n¹³, the specialist is required to possess up-to-date knowledge

and practical skills in resuscitation and intensive care. This includes the ability to:

- perform cardiopulmonary resuscitation in cases of sudden cardiac and respiratory arrest;
- manage comatose and allergic states;
- address the consequences of electrical injuries and drowning.

The physician must be proficient in securing airway patency using alternative techniques and master the protocols for the diagnosis and emergency management of:

- cardiovascular and respiratory diseases;
- pathology of the abdominal organs;
- endocrine and infectious diseases;
- mental disorders and allergic reactions.

Furthermore, the health post specialist is required to:

- know the fundamentals of medical triage in mass casualty incidents;
- perform electrocardiogram (ECG) acquisition and interpretation;
- determine blood glucose levels using portable devices;
- utilize pulse oximetry for assessing respiratory function.

Practical activities of such specialists require them to:

- safely operate medical equipment and handle medical gases;
- administer medications, including narcotic and potent drugs, considering indications, contraindications, and potential side effects;
- apply modern algorithms for the pharmacological treatment of cardiac arrest;
- perform tracheobronchial tree suction;
- utilize oxygen therapy methods;
- perform local anesthesia, primary wound care, control of external hemorrhage, and anterior nasal packing for epistaxis;
- master the techniques of urinary bladder catheterization, gastric lavage via tube, application of an occlusive dressing for pneumothorax, and aspiration of air from the pleural cavity.

A crucial element of competencies is the ability to perform immobilization for bone fractures, spinal injuries, and crush syndrome¹⁴ [13].

⁷ Order of the Ministry of Health and Social Development of the Russian Federation No. 541n "On the approval of the unified qualification directory of positions of managers, specialists, and other employees, section 'qualification characteristics of positions of workers in healthcare'" dated 23.07.2010. URL: <https://base.garant.ru/12178397/?ysclid=mg54n0gt8m637337655>

⁸ Order of the Ministry of Labor and Social Protection of the Russian Federation No. 470n "On the approval of the professional standard 'Paramedic'" dated 31.07.2020. URL: <https://base.garant.ru/74561326/?ysclid=mg54gg7rz1942350327>

⁹ Order of the Ministry of Labor and Social Protection of the Russian Federation No. 293n "On the approval of the professional standard 'General Practitioner (district physician)'" dated 21.03.2017. URL: <https://base.garant.ru/71648500/?ysclid=mg54v0od59747838905>

¹⁰ Order of the Ministry of Labor and Social Protection of the Russian Federation No. 743n "On the approval of the professional standard 'surgeon'" dated 26.11.2018. URL: <https://base.garant.ru/72125210/?ysclid=mg54y05six259152912>

¹¹ Order of the Ministry of Health of the Russian Federation No. 911n "On the approval of the procedure for providing medical care for acute and chronic occupational diseases" dated 13.11.2012. URL: <https://base.garant.ru/70290544/?ysclid=mg552x1via951179129>

¹² Order of the Ministry of Health and Social Development of the Russian Federation No. 543n "On the approval of the regulation on the organization of primary health care for the adult population" dated 15.05.2012. URL: <https://base.garant.ru/70195856/?ysclid=mg554d4suc208371979>

¹³ Order of the Ministry of Health and Social Development of the Russian Federation No. 252n "On the approval of the procedure for delegating to a paramedic or midwife by the head of a medical organization, when organizing the provision of primary health care and emergency medical care, certain functions of the attending physician for the direct provision of medical care to a patient during observation and treatment, including the prescription and use of medicinal products, including narcotic and psychotropic medicinal products" dated 23.03.2012. URL: <https://base.garant.ru/70170588/?ysclid=mg555ye84c234152130>

¹⁴ Bagnenko SF, ed. Emergency medical care. Clinical guidelines. Moscow: GEOTAR-Media; 2022.

The professional duties of medical personnel at off-shore health posts extend beyond therapeutic and diagnostic activities. The physician is responsible for:

- proper storage of medications and equipment;
 - maintaining medical documentation, including confidential and personal data;
 - organizing sobriety checks for workers and preparing corresponding official reports;
 - processing documentation for temporary disability.
- Additionally, the physician ensures:
- the correct disposal of medical waste;
 - possesses personal computer skills;
 - monitors the sanitary-hygienic and anti-epidemic condition of production and residential premises, including catering facilities and shift camps.

Regular completion of occupational health and safety briefings and knowledge assessments in this area are also an important part of their professional activity.¹⁵

The health post physician must be proficient in differential diagnosis techniques and provide qualified medical care for injuries, poisonings, and other emergency conditions [19–21]. They are responsible for monitoring the inventory of necessary medical supplies at the health post, recognizing the symptomatology of diseases related to long-term exposure to occupational hazards, and facilitating their early detection. The set of required competencies includes organizing the evacuation of casualties to medical facilities, arranging consultations with specialists via communication means, and facilitating the hospitalization of patients in specialized departments¹⁶ [21].

Planning and organizing therapeutic and preventive work holds a significant place. The physician develops an annual operational plan for the health post, ensures its implementation, conducts activities for the early diagnosis and treatment of general and occupational diseases, and analyzes the structure of morbidity and injuries. On this basis, the specialist submits proposals for reducing occupational risks and developing preventive programs. The specialist also conducts dynamic monitoring of workers' health, assesses the quality of medical examinations, and participates in bringing working conditions into compliance with sanitary standards [22].

Thus, the qualification requirements for medical personnel at health posts integrate formal legal norms, clinical competencies, and organizational tasks. The physician at an industrial health post acts not only as a

clinician but also as a manager of the medical process, an analyst of morbidity, and a participant in the occupational health and safety system. The versatility of their training and the continuous improvement of practical skills become key conditions for ensuring worker health and maintaining industrial safety.

Additional requirements, skills, and competencies for medical personnel

In the current context of providing medical care at industrial enterprise health posts, the systematic additional training of medical personnel is of particular importance. A physician working in remote and resource-limited settings is obliged not only to meet formal qualification requirements but also to regularly confirm their level of practical skills through specialized training. International and domestic experience indicates that simulation-based courses are a key tool for maintaining professional readiness. These courses provide the opportunity to replicate clinical scenarios and practice action algorithms for life-threatening conditions [23].

A mandatory requirement for health post physicians must be training at accredited educational centers with a frequency of at least once every two years. The result of completing such training is the receipt of a certificate or credential, which, while not a state-issued document, confirms the mastery of modern algorithms for providing emergency care at the pre-hospital stage. This form of continuing education ensures the maintenance and updating of knowledge in accordance with current clinical guidelines and international standards [2].

Of particular importance is the foundational level of training, represented by courses in life support, specifically the “Basic Cardiopulmonary Resuscitation” program.¹⁷ This training entails mastering techniques for basic cardiopulmonary resuscitation, the safe use of an automated external defibrillator, as well as algorithms for providing first aid in cases of airway obstruction and drowning. The duration of such training is typically up to eight academic hours, serving as a minimum competency requirement for a health post physician.

A more preferable direction is advanced training within the “Advanced Resuscitation Measures” course^{18,19,20} [24]. These programs develop skills in advanced cardiopulmonary resuscitation as part of a physician–nursing team, teach the differentiation and treatment of

¹⁵ Decree of the Government of the Russian Federation No. 2464 “On the Procedure for Occupational Health and Safety Training and Testing Knowledge of Occupational Health and Safety Requirements” dated 24.12.2021. URL: <https://base.garant.ru/403324424/?ysclid=mi04bcq88n710340032>

¹⁶ Zakrevsky YuN. Substantiation of a system for providing medical care and treatment to victims of maritime disasters: abstract of a dissertation for the degree of Doctor of Medical Sciences. Arkhangelsk, 2013.

¹⁷ Glushchenko VA, Golovanov NB, et al. Basic Cardiopulmonary Resuscitation: a textbook for students in secondary, higher and continuing professional education systems. — St. Petersburg: Petrov National Medical Research Center of Oncology; 2022.

¹⁸ Practical Guidelines / European Resuscitation Council Guidelines 2025. Advanced cardiopulmonary resuscitation in adults. URL: <https://airhab.ru/wp-content/uploads/2025/11/ALS-2025.pdf>

¹⁹ Order of the Ministry of Health of the Russian Federation No. 908n “On the procedure for establishing the diagnosis of human brain death” dated 25.12.2014 (registered with the Ministry of Justice of the Russian Federation on 12.01.2015, No. 37230, entered into force on 01.01.2016). URL: <https://base.garant.ru/71021232/?ysclid=mhww8d4tv688936936>

²⁰ Resolution of the Government of the Russian Federation No. 950 “On the approval of the rules for determining the moment of a person's death, including the criteria and procedure for establishing a person's death, the rules for terminating resuscitation measures, and the form of the protocol for establishing a person's death” dated 20.09.2012. URL: <https://base.garant.ru/70231774/?ysclid=mhww92ukww179218179>

life-threatening arrhythmias, the interpretation of ECG monitoring, and the application of modern methods for securing airway patency. The duration of such training in this case is up to 16 hours, enabling the integration of the physician into interdisciplinary pre-hospital care protocols.

An integral component of training for physicians at health posts of remote industrial facilities is mastering trauma care algorithms within the course entitled "Advanced Pre-Hospital Trauma Care."²¹ This course equips specialists with competencies for stabilizing the condition of trauma patients, ensuring control over vital functions, and preparing for medical evacuation. The program's practical focus and its duration of up to 16 hours, preparing the health post physician to act effectively in situations involving polytrauma and mass casualty incidents.

Furthermore, the mandatory training program includes courses on:

- medical escort of patients during evacuation by air transport;
- training in methods of ultrasound diagnostics for superficial tissues and fluid accumulations in body cavities.

These skills become particularly critical in the absence of specialized consultants and the need for rapid decision making at an isolated facility.

In accordance with licensing requirements, a health post physician must possess additional certifying competencies, confirmed by valid certificates. Mandatory certifications include:

- the right to conduct medical examination for intoxication;
- the right to conduct pre-shift and post-shift (pre-trip and post-trip) medical examinations²²;
- the right to perform temporary disability assessment²³.

Thus, the regular completion of additional educational programs and simulation training equips health post physicians with a comprehensive set of professional and organizational competencies that align with international standards for delivering medical care in remote settings. Systematic continuing education is a key element in ensuring the safety of industrial enterprise workers and a factor in reducing the risks of adverse outcomes in acute and critical conditions.

Introductory course for a health post physician

In the setting of offshore facilities operating in remote areas with limited transport accessibility, particularly in

the Arctic Shelf waters, medical care acquires distinct features. The inability to rapidly evacuate patients, the limited resources of health posts, and the high probability of emergencies create a demand for special training of medical professionals. To ensure their readiness to work in extreme conditions, it is advisable to conduct an additional "Introductory Course."²⁴ This course supplements the basic qualification requirements and develops the specific competencies necessary for autonomous practice.

The "Introductory Course" for a health post physician assigned to an offshore structure or vessel is a mandatory preparation stage before commencing work. This program has been developed by specialists in the field of industrial medicine, based on more than 25 years of experience in organizing the healthcare system and providing medical care at remote and hard-to-reach industrial facilities. Practice demonstrates that this course enables the physician not only to refresh theoretical knowledge and practical skills but also to gain the confidence necessary for rapid adaptation to the specifics of safeguarding the health of personnel on offshore platforms and vessels. The program's primary objective is to ensure the physician's readiness for:

- monitoring workers' professional fitness based on health status;
- preventing chronic non-communicable diseases;
- organizing emergency medical response in autonomous conditions.

The content of the "Introductory Course" is based on an assessment of worker health risks and professional standards, ensuring its alignment with current qualification requirements. The training duration is 92 academic hours, of which 49 hours are allocated to theoretical studies and 43 hours to practical skill development. The thematic plan covers a wide range of disciplines and ensures comprehensive training that integrates clinical, organizational, and sanitary-preventive competencies (Table 1).

The thematic plan encompasses a wide range of disciplines, reflecting the multifunctional nature of a physician's activity at an offshore facility. The theoretical part covers the fundamentals of industrial medicine, regulatory frameworks for medical practice, prevention of chronic non-communicable and infectious diseases, assessment of professional fitness, specifics of mental disorders and addictions, as well as aspects of sanitary and epidemiological control [13, 18]. The practical part is focused on developing clinical and organizational skills, such as performing resuscitation procedures, ensuring airway patency, providing care for trauma and bleeding,

²¹ Guidelines for trauma emergency care. World Health Organization. URL: <https://thanatoradiology.ru/wp-content/uploads/2020/10/по-неотложной-помощи-при-травмах.pdf>

²² Order of the Ministry of Health of the Russian Federation No. 835n "On the approval of the procedure for conducting pre-shift, pre-trip, and post-shift, post-trip medical examinations" dated 15.12.2014. URL: <https://www.garant.ru/products/ipo/prime/doc/70880038/?ysclid=mi08d0gffs396757788>

²³ Order of the Ministry of Health of the Russian Federation No. 195n "On the approval of the procedure for conducting temporary disability examinations" dated 11.04.2025. URL: <https://www.garant.ru/products/ipo/prime/doc/411912828/?ysclid=mi08f0rwnw487672576>

²⁴ Methodological recommendations for medical support of personnel on marine vessels and structures in the Arctic. Methodological document of Gazprom Neft Shelf LLC. 2025.

Table. Thematic plan of the Introductory Course for a health post physician at an offshore facility

No	Module (Topic)	Theory, h	Practice, h
1	Industrial medicine and occupational health	8	–
2	Prevention of non-communicable and infectious diseases	7	1
3	Prevention of circulatory system diseases, critical conditions	4	4
4	Upper respiratory tract diseases, ARI/ARVI, ENT pathology, respiratory support	4	4
5	Diabetes mellitus at a remote industrial site	2	1
6	Allergic reactions (immediate and delayed hypersensitivity)	2	3
7	Surgical pathology, trauma	1	7
8	Bandaging techniques and primary surgical treatment	1	7
9	Physician role as the leader in a team with medical assistants	4	4
10	First aid training ²⁵ and health education work	2	2
11	Transportation and immobilization	1	7
12	Mental disorders, addictions	2	2
13	Anti-alcohol policy	7	1
14	Health post equipment registers, pharmacopoeia, ship's first aid kit, narcotic analgesics, turnover	6	2
15	Industrial sanitary control and hygiene	2	2

Table adapted by the authors from the regulatory document²⁶

executing immobilization and transportation of casualties²⁷, and using personal protective equipment and medical devices [16, 25].

Particular emphasis is placed on preparation for managing critical conditions, including acute coronary syndrome, cardiac arrhythmias, severe allergic reactions, and polytrauma [25]. Independent modules are dedicated to providing care for respiratory tract diseases, glycemic control in diabetes mellitus, and managing patients under the influence of alcohol or drugs. The program also includes fundamentals of minor surgery and bandaging techniques, which is especially important when immediate evacuation is impossible.

An important component of the course is the development of leadership and teamwork skills. The health post physician typically works alongside medical assistants, thus being responsible for organizing adequate medical response in emergency situations. Practical sessions practice teamwork algorithms, medical triage in mass casualty incidents, and training enterprise workers in elements of first aid. Such preparation develops the physician's confidence in their own professional competence and readiness to coordinate staff actions in extreme situations [26].

An equally significant area comprises modules related to preventive work. The physician is trained to conduct dynamic monitoring of the worker health status, implement anti-alcohol programs, and supervise the sanitary conditions of living quarters, catering, and water supply. These aspects are particularly relevant for isolated groups, where infectious diseases and behavioral risks can rapidly escalate into a serious threat.

The "Introductory Course" not only reinforces the existing knowledge but also adapts this knowledge to the specifics of a remote offshore structure. As a result, the physician develops a specific set of competencies beyond standard training, allowing them to become a key figure in the entire system of medical safety at a particular industrial facility.

Thus, the "Introductory Course" serves as a bridge between mandatory and additional competencies, on the one hand, and the practice of autonomous work at Arctic offshore structures, on the other. Its completion becomes a criterion of the specialist's readiness for work in remote conditions, a confirmation of the ability to combine clinical, organizational, and preventive activities, and a guarantee for the employer in ensuring personnel health and safety. This is why successful completion of

²⁵ Order of the Ministry of Health of the Russian Federation No. 220n «On the approval of the procedure for rendering first aid» dated 03.05.2024. URL: <https://www.garant.ru/products/ipo/prime/doc/409012510/?ysclid=mi04kavix797820509>

²⁶ Guidelines for medical support of personnel on marine vessels and structures in the Arctic. Methodological document of Gazprom Neft Shelf LLC. 2025.

²⁷ Baranova NN. Medical evacuation of casualties in emergency situations: dissertation for the degree of Doctor of Medical Sciences. Moscow, 2022. [In Russ.].

the course is considered an essential requirement for a physician to be approved for work at offshore platforms and support vessels [27].

Additional training and professional licensing requirements for healthcare professionals

To perform duties in remote industrial settings, including offshore structures and support vessels, medical personnel are required to undergo specialized training and possess verified documentation, which is an essential prerequisite for professional authorization.

For physicians working on emergency rescue duty vessels and at health posts of offshore ice-resistant fixed platforms, the following requirements are mandatory:

- a certificate in Basic Offshore Safety Induction and Emergency Training (BOSIET), or
- a certificate in Helicopter Underwater Escape Training (HUET), valid for at least one year from the commencement of medical service provision.

Additionally, a physician on an emergency rescue duty vessel must possess:

- a Seafarer's Identity Card,
- a Seaman's book, and
- a Medical Fitness Certificate for Seafarers, which confirms professional suitability for service in maritime conditions.

For physicians at health posts of offshore platforms, completion of training in the use of an evacuation slide (Sky Shute/Sky Scape) is mandatory, also with a certificate valid for at least one year.²⁸

In addition, medical personnel are required to possess a valid health record book and an official medical certificate of professional fitness for work, issued in accordance with the current legislation of the Russian Federation. An important requirement is undergoing mandatory psychiatric examination with a conclusion from a specialized commission confirming the absence of mental disorders specified in the Decree of the Government of the Russian Federation.²⁹ This requirement applies to specialists working in the oil and gas industry, including those on a rotational basis in the Far North regions, as well as to employees whose activities involve the handling of narcotic and psychotropic substances and work in emergency medical services.

Mandatory admission conditions also include immunization of medical personnel according to the national and regional vaccination schedules, including vaccination against hepatitis B. This set of measures ensures not only the legitimacy of a physician's admission to perform professional duties but also their actual readiness to work under conditions of high professional load, elevated risks, and limited access to specialized medical care.³⁰

CONCLUSION

The conducted analysis has confirmed that training of medical personnel for health posts at offshore Arctic facilities remains one of the key unresolved tasks in the Russian healthcare system. The existing education system does not ensure the training of specialists capable of autonomous work in conditions of limited resources and prolonged transport inaccessibility.

The staffing insufficiency is exacerbated by the absence of a professional standard for a "shipboard (platform) physician," which hinders the creation of a stable personnel reserve for strategically important industrial facilities. The existing mandatory qualification requirements only allow for minimal confirmation of professional readiness and do not cover the entire range of competencies necessary for work in Arctic conditions.

Under these conditions, additional training becomes particularly important, including regular simulation trainings (BLS, ALS, ITLS) and specialized programs, as well as an "Introductory Course" that integrates clinical and organizational skills, taking into account the specific features of working at Arctic offshore structures. Such a multi-level training model equips physicians with competencies that go beyond standard requirements and fosters their readiness for patient management in autonomous conditions.

The final element in developing the medical support system should be the creation of a unified registry of physicians who have completed the full training program. This tool will enhance the transparency of personnel policy, simplify the selection of specialists for work at remote facilities, and ensure an adequate level of medical safety in the Arctic Zone of the Russian Federation.

²⁸ National Standard of the Russian Federation GOST R 54382-2021 "Oil and gas industry. Subsea pipeline systems. General technical requirements" (approved and enacted by Order No. 403-st of the Federal Agency for Technical Regulation and Metrology dated 20.05.2021).

²⁹ Decree of the Government of the Russian Federation No. 377 dated 28.04.1993 "On the implementation of the Law of the Russian Federation 'On psychiatric care and guarantees of citizens' rights in its provision'".

³⁰ Order of the Ministry of Health of the Russian Federation No. 1122n "On the approval of the national calendar of preventive vaccinations, the calendar of preventive vaccinations for epidemic indications, and the procedure for carrying out preventive vaccinations" dated 06.12.2021.

References

1. Bumay OK, Abakumov AA, Turlakov YS. Topical issues of creating a unified system of medical support for ships in the water area of the Northern Sea Route. *Extreme Medicine*. 2020;22(1):109–16 (In Russ.).
2. Bukhtiyarov IV, Golovkova NP, Khelkovsky-Sergeev NA, Kotova NI. Current issues of occupational medicine in the Russian fishing industry. *Occupational Health and Industrial Ecology*. 2018;12:24–8 (In Russ.).
<https://doi.org/10.31089/1026-9428-2018-12-24-28>
3. Voronkova SV, Abakumov AA, Bumay OK, Grabsky YV, Torshin GS, Malinina SV, et al. Current state and problematic issues of medical care in the water area of the Northern Sea Route. *Occupational Health and Industrial Ecology*. 2022;62(4):212–24 (In Russ.).
<https://doi.org/10.31089/1026-9428-2022-62-4-212-224>
4. Ibragimov IRO. On the use of marine autonomous surface vessels in relation to ensuring the safe activities of seaports. *Ocean Management*. 2023;4(22):20–4 (In Russ.).
5. Shevchenko KV, Dunayeva EA, Ladilova YD. Accessibility of healthcare in the Arctic zone of Russia. *Agrarian and Land Law*. 2023;9(225):7–10 (In Russ.).
6. Shenshin VM, Nazarova IS, Utkin NI. On the role of the Ministry of Emergency Situations of Russia in the national security system in the Arctic zone of the Russian Federation. *Law and State: Theory and Practice*. 2023;5(221):50–5 (In Russ.).
7. Shenshin VM, Platonov KM. Ensuring the national security of the Russian Federation in the Arctic and the socio-economic development of the Arctic zone of the Russian Federation as a priority area of ensuring Russia's national interests in the Arctic. *Security Service in Russia: Experience, Problems, Prospects. The Arctic — a region of strategic interests: legal policy and modern technologies for ensuring security in the Arctic region*. Proceedings of the International Scientific and Practical Conference. St. Petersburg; 2022 (In Russ.). EDN: [UXCOLD](#)
8. Yakovleva TV, Turenko OY, Ratnikov VA, Moskaleva SS. Key results and directions for improving medical support of seafarers within the Federal Medical-Biological Agency of Russia: a retrospective study. *Marine Medicine*. 2024;10(4):71–83 (In Russ.).
9. Levanov VM, Perevezentsev EA. Possibilities of complex use of telemedicine technologies in the system of medical support for the working population in remote territories (literature review). *Bulletin of New Medical Technologies. Electronic Edition*. 2019;1:102–10 (In Russ.).
10. Orlov OI, Mamonova EY, Levanov VM, Kalinina MY. Issues of organizing remote training for medical staff of remote medical stations on emergency medical care. *Siberian Herald of Medical Informatics and Healthcare Informatization*. 2016;1:66–9 (In Russ.). EDN: [YLKENR](#)
11. Orlov OI, Mamonova EY, Vasilyev IM, Romanov MA, Kalinina MY. Risk-based approach to the organization of work in extreme conditions on an offshore oil platform in the Arctic shelf area. *Aerospace and Environmental Medicine*. 2025;58(1):111–7 (In Russ.).
<https://doi.org/10.21687/0233-528X-2025-59-3-57-67>
12. Levanov VM, Nikonov AY, Mamonova EY, Perevedentsev OV. Remote training in continuous medical education. *Journal of Telemedicine and e-Health*. 2015;1(1):42–5 (In Russ.).
13. Karpov AB, ed. *Remote Healthcare*. Moscow: GEOTAR-Media; 2024 (In Russ.).
14. Zakrevsky YN, Manuylov VM, Matveev RP. Structure of injuries in survivors and those killed in maritime disasters. *Military Medical Journal*. 2010;331(11):42–8 (In Russ.).
15. Thibodaux DP, Bourgeois RM, Loeppke RR, Konicki DL, Hymel PA, Dreger M. Medical evacuations from oil rigs off the Gulf Coast of the United States from 2008 to 2012: reasons and cost implications. *Journal of Occupational and Environmental Medicine*. 2014;56(7):681–5.
<https://doi.org/10.1097/JOM.0000000000000221>
16. Arkhangelsky DA, Zakrevskiy YuN, Rybnikov VYu. Medical evacuation of patients (victims) in the Arctic zone by emergency formations of the Disaster Medicine Service of the Northern Fleet of Russia. *Medical-Biological and Socio-Psychological Problems of Safety in Emergency Situations*. 2019;4:27–33 (In Russ.).
17. Arkhipovsky VL. On medical support of the personnel of the Prirazlomnaya offshore ice-resistant stationary platform from 2011 to 2014. *Marine Medicine*. 2019;5(1):61–5 (In Russ.).
18. Torshin GS, Bumay OK, Malinina SV. Analysis of regulatory documents in the field of marine and diving medicine. *Extreme Medicine*. 2020;22(1):104–8 (In Russ.).
19. Gorbachev VI, Itygilov MY. Some regulatory aspects of tracheostomy. *Russian Journal of Anaesthesiology and Reanimatology*. 2019;5:22–9 (In Russ.).
<https://doi.org/10.17116/anaesthesiology201905122>
20. Gunina MV, Kadyshchev VA, Tsarapkin GY, Bulychov SN, Goncharova NA, Voronkova NY, et al. Optimization of methods of epistaxis treatment in the working conditions of ambulance and emergency medical teams. *Russian Rhinology*. 2023;31(4):287–91 (In Russ.).
<https://doi.org/10.17116/rosrino202331041287>
21. Zakrevsky YN, Matveev RP. Current problems of organizing search, rescue and assistance to victims of maritime disasters. *Human Ecology*. 2011;3:33–9 (In Russ.).
22. Kovalev SP, Yashina ER, Ushakov IB, Turzin PS, Lukichev KE, Generalov AV. Corporate programs for strengthening occupational health of workers in the Russian Federation. *Human Ecology*. 2020;10:31–7 (In Russ.). EDN: [RTXUYQ](#)
23. Barbachuk FM, Biderman FM, Boykov AA, Grebennikov VA, Goryacheva AG, Emelyanova AV, et al. *Algorithms for providing emergency medical care outside a medical organization: a guide for medical professionals working in emergency medical teams*. St. Petersburg: IP Shevchenko VI; 2018 (In Russ.).
24. Kuzovlev AN. Advanced resuscitation measures in cardiac arrest: current guidelines and prospective research. *Bulletin of Intensive Care*. 2017;3:55–61 (In Russ.).
25. Labardin AM. Preparing rescuers for action in the Arctic region. *Problems of preventing and responding to emergency situations in the Arctic, including issues of training specialized personnel to work in northern conditions*. Proceedings of the International Conference. Moscow, 2014 (In Russ.). EDN: [INTPKX](#)
26. Goncharov SF, Baranova NN, Starkov SA, Starkov AS. Medical evacuation in the Arctic region: problems and solutions. *Kremlin Medicine. Clinical Bulletin*. 2024;1:70–4 (In Russ.). EDN: [IKLGXD](#)
27. Razletova AB, Nikitina VN, Lyashko GG, Kalinina NI, Dubrovskaya EN, Kotenko PK. Hygienic assessment of the features of the crew's labor activity in the Arctic conditions and regulatory support for the protection of sailors' health. *Marine Medicine*. 2022;8(3):88–99 (In Russ.).

Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria and approve the final version of the article. The primary contributions are distributed as follows: Oleg I. Orlov — supervision and guidance of research planning and execution, methodological control, approval of the work's structure and content, manuscript editing, and final approval of the article version.; Elena Yu. Mamonova — formulating the concept, research aims and objectives, developing the methodology and creating the research model, participating in data interpretation and preparing the analytical sections of the article, coordinating the work of the author team; Michael A. Romanov — collection, analysis, and synthesis of literature data; Maria Yu. Kalinina — analysis of legislative and regulatory-methodological frameworks, systematization of the obtained data, manuscript editing.

AUTHORS

Oleg I. Orlov, Dr. Sci. (Med.), Professor, Academician of RAS

<https://orcid.org/0000-0001-8429-1076>
olegtm@bk.ru

Elena Yu. Mamonova, Cand. Sci. (Med.)

<https://orcid.org/0009-0001-2612-8710>
elenamamonova@mail.ru

Michael A. Romanov, Cand. Sci. (Med.)

<https://orcid.org/0009-0005-8749-676X>
romanov.ma@gazprom-neft.ru

Maria Yu. Kalinina, Cand. Sci. (Med.)

<https://orcid.org/0000-0002-8798-5732>
MYKalinina@rosatom.ru

<https://doi.org/10.47183/mes.2025-375>



MULTISTAGE SURGICAL MANAGEMENT OF PATIENTS WITH COMBINED CRANIOFACIAL INJURIES

Yurii A. Shcherbuk¹, Olga D. Madai², Dmitrii Yu. Madai^{2,3,✉}, Alexandr Yu. Shcherbuk⁴

¹ Kirov Military Medical Academy, St. Petersburg, Russia

² St. Petersburg State University, St. Petersburg, Russia

³ St. Petersburg Dzhanelidze Research Institute of Emergency Medicine, St. Petersburg, Russia

⁴ Peoples' Friendship University of Russia, Moscow, Russia

Introduction. Recent observations indicate that craniofacial injuries can present as isolated, multiple, or combined trauma. Currently, there is a lack of objective criteria for determining impairments of vital functions that necessitate specialized, multidisciplinary medical care at Level I trauma centers, including multistage surgical treatment (MST) as a criterion for comprehensive management. Furthermore, criteria and indications for the comprehensive treatment of combined injuries, which account for up to 80% of cases, have not been developed in sufficient detail.

Objective. Development of a rational MST protocol for combined trauma that takes into account injury severity and impaired body functions in patients with midface injuries.

Materials and methods. A total of 132 patients were examined, with males comprising 84.8% ($n = 112$) and females comprising 15.2% ($n = 20$). The age distribution of the patients was as follows: males aged 20–39 years — 44.7% ($n = 59$), males aged 40–59 years — 40.1% ($n = 53$); females aged 20–39 years — 1.5% ($n = 2$), females aged 40–59 years — 13.6% ($n = 18$). All patients were divided into three groups: Group 1 — 40 patients (30 males; 10 females) with a compensated course (trauma disease (TD) Type I); Group 2 — 44 patients (38 males; 6 females) with a subcompensated course (TD Type II); Group 3 — 48 patients (44 males; 4 females) with a decompensated course (TD Type III). The study outcomes were assessed based on injury severity and impaired body functions. Injury severity was evaluated using the Trauma Shock Potential Scale (Tsibin), the Military Field Surgery — Injury Scale (MFS-I), and the Abbreviated Injury Scale (AIS). Impaired body functions were evaluated using MFS-SE (State Upon Entry), MFS-SA (State After Admission), and MFS-SS (Specialized State) scales. Statistical data processing was performed using the Statistica 10 software package. Standard descriptive statistics were calculated (mean [M], standard deviation [SD], median [M_e], lower [Q_1] and upper [Q_3] quartiles). Hypothesis testing for the equality of means was assessed using Student's t -test.

Results. The conducted analysis of statistical data on the nature of injuries and impaired vital body functions allowed an MST methodology to be developed. Its application enabled adequate administration of specialized resuscitative care in 90% of cases, as well as multidisciplinary surgical care. For Group 1 patients, MST was primarily provided on an abbreviated basis, without performing a tracheostomy or gastrostomy, followed by a full-scale surgical intervention during the first period of TD. Treatment for patients in Groups 2 and 3 was initiated in a shock room or an intensive care unit (ICU).

Conclusions. In cases of TD Type I, i.e., isolated maxillofacial injury with a compensated course, the principles and algorithms of the general therapeutic and tactical approach to TD management are not applicable, and the full scope of surgical interventions can be performed during the first period of TD. In cases of TD Types II and III, the full scope of MST should be implemented.

Keywords: combined trauma; “Military Field Surgery — Injury” scale; “Military Field Surgery — State Upon Entry” scale; “Military Field Surgery — State After Admission” scale; multistage surgical treatment

For citation: Shcherbuk Yu.A., Madai O.D., Madai D.Yu., Shcherbuk A.Yu. Multistage surgical management of patients with combined craniofacial injuries. *Extreme Medicine*. 2026;28(1):123–133. <https://doi.org/10.47183/mes.2025-375>

Funding: the study was conducted without external funding.

Compliance with ethical principles: the study was conducted in accordance with the principles of the Declaration of Helsinki. The clinical study protocol was approved by the Independent Ethics Committee of the St. Petersburg Dzhanelidze Research Institute for Emergency Medicine (Minutes No. 925 of 14.03.2024). All patients and/or their legal representatives provided written informed consent for treatment and the use of anonymized medical data and photographs.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Dmitrii Yu. Madai wpxmdy@mail.ru

Received: 13 Aug. 2025 **Revised:** 20 Oct. 2025 **Accepted:** 7 Nov. 2025 **Online first:** 27 Dec. 2025

УДК 616.08

МНОГОСТАДИЙНОЕ ХИРУРГИЧЕСКОЕ ЛЕЧЕНИЕ ПОСТРАДАВШИХ С СОЧЕТАННЫМИ ЧЕРЕПНО-ЛИЦЕВЫМИ ПОВРЕЖДЕНИЯМИ ГОЛОВЫ

Ю.А. Щербук¹, О.Д. Мадай², Д.Ю. Мадай^{2,3,✉}, А.Ю. Щербук⁴

¹ Военно-медицинская академия им. С.М. Кирова МО РФ, Санкт-Петербург, Россия

² Санкт-Петербургский государственный университет, Санкт-Петербург, Россия

³ Санкт-Петербургский НИИ скорой помощи им. И.И. Джanelидзе, Санкт-Петербург, Россия

⁴ Российский университет дружбы народов им. Патриса Лумумбы, Москва, Россия

© Yu.A. Shcherbuk, O.D. Madai, D.Yu. Madai, A.Yu. Shcherbuk, 2025

Введение. В последнее время наши наблюдения показали, что травмы черепно-лицевой области протекают в виде изолированных, множественных и сочетанных травм. До настоящего момента нет объективных критериев нарушения жизненно важных функций организма, при которых пострадавшие нуждаются в специализированной многопрофильной медицинской помощи в травмоцентрах I уровня, в том числе и в виде многостадийного хирургического лечения (МСХЛ) как критерия комплексности. Не разработаны критерии и показания к комплексному лечению сочетанных травм, частота которых достигает 80%.

Цель. Разработка рационального МСХЛ сочетанных травм с учетом тяжести повреждения и нарушенных функций организма пострадавших с травмой средней зоны лица (СЗЛ).

Материалы и методы. Обследовано 132 пострадавших, среди них мужчины составили 84,8% (112 пострадавших), женщины — 15,2% (20 пострадавших). По возрасту пострадавшие распределены следующим образом: мужчины в возрасте 20–39 лет — 44,7% (59 пациентов), в возрасте 40–59 лет — 40,1% (53 пациента); женщины в возрасте 20–39 лет — 1,5% (2 пациентки), в возрасте 40–59 лет — 14% (18 пациенток). Все пострадавшие были разделены на 3 группы: группа 1 — 40 пациентов (30 мужчин; 10 женщин) с компенсированным вариантом течения (I вариант течения травматической болезни (ТБ)); группа 2 — 44 пациента (38 мужчин; 6 женщин) — субкомпенсированный вариант (II вариант течения ТБ); группа 3 — 48 пациентов (44 мужчины; 4 женщины) — декомпенсированный вариант (III вариант течения ТБ). Результаты исследования оценивали по показателям тяжести повреждений (с использованием шкалы шокогенности травмы Ю.Н. Цыбина, ВПХ-П, AIS) и нарушенных функций организма (ВПХ-СП, ВПХ-СГ, ВПХ-СС). Статистическая обработка данных проведена с использованием пакета программного обеспечения Statistica 10. Вычисляли стандартные описательные статистики (среднее значение (M), стандартное отклонение (δ), медиану (M_e), нижний (Q_1) и верхний (Q_3) квартили). Проверка гипотезы о равенстве средних значений оценена с использованием t -критерия Стьюдента.

Результаты. Изучение результатов статистического анализа характера повреждений и нарушенных жизненно важных функций организма позволило разработать методику МСХЛ. Ее использование позволило адекватно применять специализированную реаниматологическую помощь в 90% случаев и многопрофильную хирургическую помощь. Больным 1-й группы оказывалось МСХЛ в основном в «сокращенном» варианте, без применения трахеостомии и гастростомии, а в дальнейшем выполнялось оперативное вмешательство в полном объеме в первом периоде травматической болезни. Лечение пострадавших 2-й и 3-й групп начиналось в противошоковой операционной (ПШО) или в отделении реанимации и интенсивной терапии (ОРИТ).

Выводы. При первом варианте ТБ — изолированной травме (компенсированное течение) челюстно-лицевой области — принципы и алгоритмы лечебно-тактической концепции ТБ неприемлемы, и можно выполнять весь объем хирургических вмешательств в первом периоде ТБ. При втором и третьем вариантах ТБ выполняется весь объем МСХЛ.

Ключевые слова: сочетанная травма; шкалы Военно-полевая хирургия — повреждение; Военно-полевая хирургия — состояние при поступлении; Военно-полевая хирургия — состояние госпитальное; многостадийное хирургическое лечение

Для цитирования: Щербук Ю.А., Мадай О.Д., Мадай Д.Ю., Щербук А.Ю. Многостадийное хирургическое лечение пострадавших с сочетанными черепно-лицевыми повреждениями головы. *Медицина экстремальных ситуаций*. 2026;28(1):123–133. <https://doi.org/10.47183/mes.2025-375>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование выполнено с соблюдением требований и норм Хельсинкской декларации. Проведение клинического исследования одобрено независимым этическим комитетом Санкт-Петербургского Научно-исследовательского института скорой помощи им. И.И. Джанелидзе (протокол № 925 от 14.03.2024). Все пациенты и/или их родственники подписали информированное согласие на лечение и использование обезличенных медицинских данных и фотографий.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Мадай Дмитрий Юрьевич wpxmdy@mail.ru

Статья поступила: 13.08.2025 **После доработки:** 20.10.2025 **Принята к публикации:** 07.11.2025 **Online first:** 27.12.2025

INTRODUCTION

Multistage surgical treatment (MST) is the cornerstone of management for patients with midface injuries. This approach is based on assessing the severity of the patient's general condition and evaluating the level of compensation for impaired body functions. The MST includes the following three stages: primary emergency surgery on a limited basis (abbreviated); intensive therapy until stabilization of vital body functions; and repeated surgical intervention to correct the remaining injuries. Currently, adequate standardized algorithms for MST are lacking [1–3].

The refinement of MST for patients with combined craniofacial trauma remains a critical challenge in trauma surgery. The relevance of this issue is underscored by the high mortality rate observed in these patients. In the

late 20th century, the Early Total Care (ETC) system was proposed, which advocated for the treatment of all injuries within the first 24 h. However, the ETC system cannot be considered versatile; moreover, its use does not lead to a reduction in mortality [4–7].

Recently, new approaches for the treatment of severe mechanical trauma based on the damage control strategy have been developed [7, 8]. Three types of this strategy can be distinguished:

- damage control tactics,¹ which involves assessing the severity of the initial trauma and the patient's biological constitution (age, body weight, comorbidities, number of required surgeries, their duration, and invasiveness);
- second-look operation tactics aimed at repeated assessment of the condition of an injured organ or pathological focus after the primary surgery with the

¹ Maday DYU. Choice of Surgical Treatment Strategy for Patients with Combined Injuries Depending on Injury Severity: Lecture. Saint Petersburg: Saint Petersburg State University Publishing House; 2010.

purpose of detecting early recurrences or evaluating the effect of therapy that could not be completed during the first operation;

- programmed relaparotomy involving a repeated surgical access through an abdominal incision, performed after the primary surgery to manage complications, disease progression, or to perform sanitation, as in case of diffuse peritonitis. It can be emergency (on-demand), triggered by a sudden deterioration of the patient's condition, or planned (scheduled), when the reintervention is pre-planned [1].

According to V.A. Sokolov, immunological studies have confirmed that trauma induces a local inflammatory response (LIR) with increased concentrations of pro-inflammatory cytokines. This cascade of changes leads to the development of a condition referred to as the Multiple Organ Dysfunction Syndrome (MODS) in the Western scientific literature. In Russian publications, it is called the Disseminated Intravascular Coagulation (DIC) syndrome.²

MST is applied when it is possible to assess the severity of trauma, the general condition of the organism, the number of surgical interventions required, and their potential outcome [1, 7–10]. Such a multistage approach is a treatment strategy of choice for critical conditions, involving only those methods which do not worsen the status of patients with varying degrees of general condition severity, as assessed based on objective indicators.

The foundation of specialized, multidisciplinary medical care for severe combined craniofacial trauma consists in resuscitative measures and multistage surgical assistance. This integrated approach enables the creation of a therapeutic and tactical scheme for comprehensive treatment, spanning from the moment of admission to a shock room through to the rehabilitation period. The advantage of this approach, in contrast to the “damage control” strategy, lies in the fact that MST is based both on damage control during the execution of Stages 1 and 2, as well as on performing restorative surgical interventions during the rehabilitation period.

At the beginning of the 21st century, the Russian healthcare system face two major challenges. These were the implementation of a new national healthcare system and the integration of a system for emergency specialized multidisciplinary medical care into regional healthcare. This new system integrates prehospital specialized care with Level I and Level II regional trauma centers to provide emergency specialized multidisciplinary medical assistance. This framework allows for the execution of specific elements of MST at Level II trauma centers (e.g., temporary control of ongoing external hemorrhage, management of asphyxia by cricothyrotomy and tracheostomy).

Severe combined trauma requires emergency specialized multidisciplinary medical care, including specialized resuscitative care in intensive care units (ICUs) and

emergency multidisciplinary specialized surgical care (MSSC) in operating rooms. Specialized resuscitative care involves addressing several objectives: (1) restoration of circulating blood volume, (2) maintenance of adequate circulation, and (3) respiratory therapy. Emergency MSSC is provided in full in shock rooms. One-third of all surgeries are performed under delayed emergency indications, while one-fifth are urgent surgeries [1]. A crucial aspect in the management of severe combined trauma is the provision of the full scope of emergency multidisciplinary specialized surgical care in the form of MSSC.

In the ICU, the Military Field Surgery — State After Admission (MFS-SA) scale is used for the dynamic assessment of the severity of physiological function impairments (i.e., the severity of the patient's condition). Subsequently, the Military Field Surgery — Specialized State (MFS-SS) scale is applied. This scale enables the diagnosis of endotoxemia, multiple organ failure, acute lung injury syndrome, and systemic inflammatory response syndrome.

In this study, we aim to develop a methodology of MST for combined midface injuries, taking into account the trauma severity and impaired body functions.

MATERIALS AND METHODS

A total of 132 patients were examined, with 84.8% ($n = 112$) of males and 15.2% ($n = 20$) of females. The age distribution was as follows: males aged 20–39 years — 44.7% ($n = 59$), males aged 40–59 years — 40.1% ($n = 53$); females aged 20–39 years — 1.5% ($n = 2$), females aged 40–59 years — 13.6% ($n = 18$). The patients were admitted from the combined trauma departments of Level I trauma centers: the Dzhanelidze Research Institute for Emergency Medicine, the Alexandrovskaya Hospital, and the Military Field Surgery Department of the Kirov Military Medical Academy.

Injury severity criteria were assessed using the following scales:

- Military Field Surgery — Injury (MFS-I) scale for the quantitative assessment of injury severity, developed at the Military Field Surgery Department of the Kirov Military Medical Academy;
- AIS (Abbreviated Injury Scale), which is a standardized scale based on the assessment of specific anatomical injuries in isolated trauma, accounting for the type of injury and its location (points), and impairments of general body functions.

The following scales were used to assess functional impairments: Military Field Surgery — State Upon Entry (MFS-SE); Military Field Surgery — State After Admission (MFS-SA); Military Field Surgery — Specialized State (MFS-SS) [1, 7].

All patients were divided into three groups:

- Group 1 — 40 patients (30 males; 10 females) with a compensated course (trauma disease (TD) Type I);

² Sokolov VA. “Damage Control” — A Modern Concept for the Management of Patients with Critical Polytrauma. Lecture. *Priorov Journal of Traumatology and Orthopedics*. 2005.

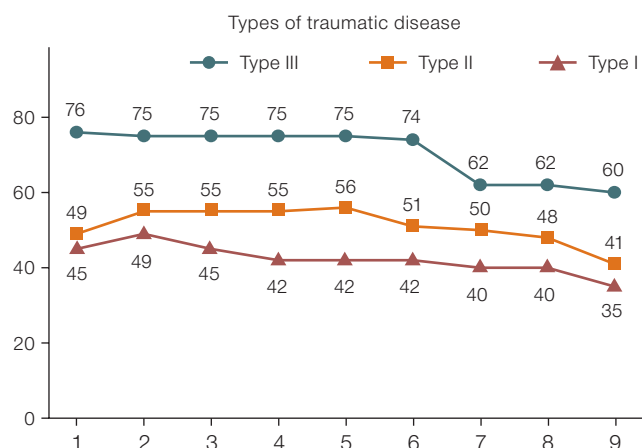


Figure prepared by the authors based on original data

Fig. 1. Daily score dynamics of the general condition of patients in the study groups according to the MFS-SS Scale (Military Field Surgery — Specialized State): X-axis — observation day; Y-axis — condition severity score (MFS-SS scale)

- Group 2 — 44 patients (38 males; 6 females) with a subcompensated course (TD Type II);
- Group 3 — 48 patients (44 males; 4 females) with a decompensated course (TD Type III).

The patients were comparable in terms of sex, age, nature of traumatic injury, structure of combined injuries, and severity of blood loss.

The study outcomes were assessed using the following parameters: injury severity (using the Trauma Shock Potential Scale (Tsibin scale), MFS-I scale, and AIS) and impaired body functions (MFS-SE, MFS-SA, and MFS-SS).

Statistical data processing was performed using the Statistica 10 software. Standard descriptive statistics were calculated (mean [M], standard error of the mean [SEM], median [M_e], lower [Q_1] and upper [Q_3] quartiles). The significance of differences in quantitative variables between independent groups was determined using Student's t -test (for normally distributed variables).

RESULTS AND DISCUSSION

During the study, in 40 patients with TD Type I (Group 1), the severity of injuries was assessed upon admission using the MFS-I scale at 1.0–2.0 points, and the degree of impaired functions was assessed at 18.8 ± 0.9 points on the MFS-SE scale. Following the performance of emergency and urgent surgical interventions, a rapid decrease in the condition severity index, calculated according to the MFS-SS methodology, was observed.

Simultaneously, in 44 patients (Group 2) with TD Type II, the second period of trauma became distinctly manifest on Day 2 after the injury. This was accompanied

by the minimum value of the condition severity index over a 10-day period (MFS-SS — 47.5 ± 0.3 points).

In 48 patients with TD Type III, the trauma disease had the most severe manifestation (complex trauma with impairment of vital functions — polytrauma). The injury severity in this subgroup ranged 19.0–45.5 points on the MFS-I scale (averaging 26.2 ± 0.8 points). The minimum severity of the condition upon admission was 32.0 points on the MFS-SE scale, and the maximum was 75.0 points (Fig. 1).

The algorithm for diagnosing and treating combined craniofacial injuries was implemented based on a standard fixed parameter (injury severity) and a variable parameter (severity of impaired body functions). Clinical criteria for patient distribution are presented in Tables 1 and 2.

Table 1 presents the criteria for patient hospitalization based on the assessment of the general condition severity using the MFS-SE and MFS-SA scales.

Table 2 presents the injury severity scores for the patients.

In accordance with the requirements of MST, we performed the following measures in the treatment of patients [8, 9]:

- establishment of upper airway patency (elimination of dislocation, obturation, stenotic, valve-type, and aspiration asphyxia);
- hemostasis, including ligation of vessels “at a distance” and tamponade of the oral cavity, oropharynx, and nasopharynx, following preventive tracheotomy and insertion of a gastric tube;
- temporary fracture fixation (Fig. 2);
- prolonged mechanical ventilation for subcompensated and decompensated TD courses;
- reconstructive and restorative surgical interventions.

The photographs (Fig. 2) show defects in the orbital, nasal, and maxillofacial regions that require the full scope of plastic and reconstructive surgical interventions to correct the sequelae of trauma during the rehabilitation period.

Comprehensive surgical treatment for combined craniofacial trauma was performed using a methodology based on the clinical and pathogenetic manifestations of TD and its stages of progression [8, 9]. When implementing the developed MST approach according to the compensation level for impaired body functions, we used the MFS-SS scale and the protocol of Military Field Surgery Department of the Kirov Military Medical Academy. According to this framework, the entire MST algorithm was to be carried out.³

At the initial stage of managing patients with combined midface injuries, the compensation level for impaired functions was assessed. Upon identifying signs of subcompensation or decompensation, MST was performed under an emergency protocol. This protocol involved providing emergency care in a shock room,

³ Ivanov A.G. Content and Organization of Surgical Care for Maxillofacial Wounds in the Context of the Special Military Operation. Study Guide. Saint Petersburg: Saint Petersburg State University Publishing House.

Table 1. Qualitative and quantitative parameters of trauma

Conventional classification of impaired functions severity	Scales	Scoring characterization of impaired functions severity	Lethality (%)	Complication rate (%)
Mild	MFS-SE MFS-SA MFS-SS	12 16–22 27–32	0 0 0	0 0 0
Moderate	MFS-SE MFS-SA MFS-SS	13–20 23–32 33–49	<3.5 <3.5 <7.5	34.0 <34.0 <27.5
Severe	MFS-SE MFS-SA MFS-SS	21–31 33–40 50–69	<38.0 <38.0 <32.0	<66.0 <66.0 <62.7
Extremely Severe	MFS-SE MFS-SA MFS-SS	32–45 41–50 70–98	<84.0 <84.0 <75.0	<90.0 <90.0 <86.3
Critical	MFS-SE MFS-SA MFS-SS	>45 >50 >99	100 100 100	– – –

Table compiled by the authors based on original data

Note: MFS-SE — Military Field Surgery — State Upon Entry; MFS-SA — Military Field Surgery — State After Admission; MFS-SS — Military Field Surgery — Specialized State.

Table 2. Injury severity scores in patients

Injury type	Scales		
	AIS	Tsibin scale	MFS-I
Soft tissue wounds of the head	1	-	0.05
Closed nasal bone fracture	1	1.5	0.2
Brain concussion	1	0.1	0.2
Jaw fractures	2	1.5	0.3
Mild cerebral contusion	2	0.1	0.3
Moderate brain contusion with calvarial fractures	2	0.5	0.5
Moderate brain contusion with closed fractures of the calvaria and skull base	3	6	0.6
Fractures of the calvaria and skull base (open type), traumatic brain injury (TBI), brain contusion	4	6	2
Brain compression associated with non-severe contusions	5	1	7
Severe brain contusion with injury to the upper brainstem	5	4	12
Brain compression on a background of severe contusions	5	-	18
Severe brain contusion with injury to the lower brainstem	5	2	19

Table compiled by the authors based on original data

Note: AIS — Abbreviated Injury Scale; MFS-I — Military Field Surgery — Injury.

Table 3. Compensation variants for impaired functions according to the Military Field Surgery Scale

Petameter	Compensated course of TD Type I	Subcompensated course of TD Type II	Decompensated course of TD Type III
Inotropic support	not administered	not administered	administered
Systolic BP (mm Hg)	100	81–100	≤ 80
HR (beats/min)	60–90	91–140	< 60 or > 140
Hematocrit (%)	31	26–31	20–25
Hemoglobin (g/L)	99	85–99	60–84
Erythrocytes (×10 ¹² /L)	2.9	2.6–2.9	2.5
MFS-SS, points	50	50–69	70–98

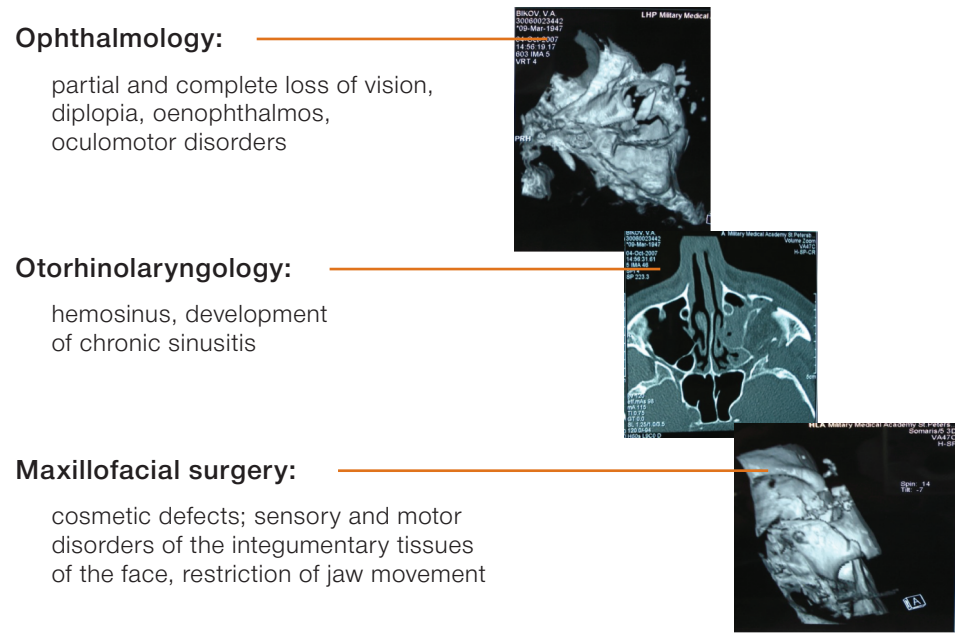
Table compiled by the authors based on original data
Note: TD — traumatic disease; BP — blood pressure; MFS-SS — Military Field Surgery — Specialized State.

followed by transfer to a specialized department for continued treatment after stabilization of the general condition. Upon admission, it is crucial to assess the nature of impaired functions [13–15]. In a decompensated course of the disease, the full scope of Stage 1 MST must be performed. In a subcompensated course of TD, the full scope of Stage 2 MST measures is required. In a compensated course of TD, surgical interventions according to Stage 3 MST can be performed immediately upon admission. Table 4 presents the variants of TD course.

The analysis of injury severity (MFS-I scale) and general condition (MFS-SE scale) revealed statistically significant differences between TD Types I, II, and III. On this basis, the use of the abbreviated MST protocol was

not required, and the full scope of surgical intervention for patients in Group 1 was performed.

In 40 patients from Group 1, following emergency and urgent surgical interventions, a rapid decrease in the condition severity index, calculated using the MFS-SS (Military Field Surgery — Specialized State) methodology, was observed. Within 12 h of hospital admission, its values did not exceed 49 points (moderate severity condition), averaging 45.8 ± 0.3 points. It should be noted that in some patients (19 patients (38.0%)) of this subgroup, the MFS-SS index was already below 49 points within 6 h of hospitalization. Starting from Day 3, the state indicators of the systems indicated persistent sub-compensation. In this subgroup of patients, relatively “non-life-threatening” complications developed during



Images prepared by the authors based on original data
Fig. 2. Anatomy of midface injuries requiring multistage surgical treatment in the rehabilitation period

Table 4. Clinical variants of trauma disease course in patients

Parameters	TD Type I (Compensated)	TD Type II (Subcompensated)	TD Type III (Decompensated)
Absolute number of patients	40 (30.3%)	44 (33.3%)	48 (36.4%)
Injury Severity by MFS-I Scale, points	2.8 ± 0.4	11.2 ± 1.0*	17.9 ± 1.3*
State Severity Upon Admission by MFS-SE Scale, points	18.8 ± 0.9	26.2 ± 0.8*	42.2 ± 3.3*
Incidence of sepsis, abs. number (%)	–	8 (18.2%)	10 (20.8%)
Incidence of septic shock, abs. number (%)	–	1 (2.5%)	5 (10.4%)
Incidence of acute lung injury syndrome, abs. number (%)	4 (10%)	5 (10.4%)	10 (20.8%)
Incidence of acute respiratory distress syndrome, abs. number (%)	–	4 (9.09%)	10 (20.8%)
Incidence of other life-threatening complications (pulmonary embolism, fat embolism syndrome, generalized fibrinolysis), abs. number (%)	–	6 (13.6%)	7 (14.6%)
Lethality, abs. number (%)	–	–	7 (14.6%)

Table compiled by the authors based on original data

Note: MFS-I — Military Field Surgery — Injury; MFS-SE — Military Field Surgery (State Upon Entry); PE — pulmonary embolism; TD — trauma disease; * — differences are statistically significant at $p < 0.05$.

the third period of TD: pneumonia, endobronchitis, dysbiosis, and general lung injury syndrome (GLIS). On average, by Day 5, the MFS-SS index in these patients did not exceed 46 points, and the major life-supporting systems were in a state of compensation. There were no lethal outcomes in this subgroup of patients. A characteristic feature of the third period of trauma disease was the maintenance of the subcompensation state of the major life-supporting systems achieved during the second period.

In 44 patients from Group 2 with TD Type II, the disease progression differed somewhat from the Type I course described above. The second period of TD in Group 2 patients also became distinctly manifested on Day 2 after the injury, which was accompanied by the maintenance of the minimum value of the MFS-SS over a 10-day period — 47.5 ± 0.3 points. In the patients of this subgroup during this TD period, the systems of gas exchange, hemodynamics, and blood most frequently remained decompensated in various combinations; decompensation of liver function and the gastrointestinal tract also persisted, or marked endotoxemia was present. The subsequent third period of TD in the patients of this subgroup was characterized not only by a persistent and prolonged increase in the severity of their condition but also by the development of life-threatening complications, such as severe sepsis (18.5%), septic shock (3.0%), acute respiratory distress syndrome (ARDS), generalized fibrinolysis (5.5%), fat embolism syndrome (1.8%), and pulmonary embolism (3.7%). The injury severity in

the patients of this subgroup ranged 3.6–14.2 points on the MFS-I scale (averaging 10.3 ± 0.3 points), while the severity of the condition upon admission ranged 24.0–31.0 points on the MFS-SE scale (averaging 27.6 ± 0.4 points) (Table 1).

In 48 patients (Type III, Fig. 1), the TD course was the most severe. According to modern concepts, the injuries sustained by all these patients fell under the definition of “polytrauma”, i.e., they were the most severe and complex types of traumas with impairment of vital functions. The injury severity in this subgroup ranged 15.0–45.5 points on the MFS-I scale (averaging 18.5 ± 1.6 points). The minimum severity of the condition upon admission was 32.0 points on the MFS-SE scale, and the maximum was 75.0 points (averaging 43.4 ± 2.9 points) (Table 1).

The main feature of the first period of TD in this subgroup was high lethality. During this period, a lethal outcome occurred in 14.6% of the patients. The second period of TD in the subgroup of patients with polytrauma was indistinct (blurred); the MFS-SS index was 72.6 ± 4.3 points, with pronounced coagulopathy determining the severity of the general condition. The major life-supporting systems remained decompensated, with the exception of compensated metabolic acidosis. The third period in this subgroup was characterized by the development of life-threatening complications such as severe sepsis (20.8%) and acute respiratory distress syndrome (20.8%) [14].

Our MST methodology (Fig. 3) was proposed after studying the clinical and laboratory parameters of the

patients involved in the study. The MST technique is based on the principles of the temporal progression of TD and the dependence of the TD type on the severity of impaired body functions. MST begins in the admission and triage department and is completed upon the discharge of the patient. Surgeons and ICU specialists face the decision regarding the necessity of performing tracheostomy and gastrostomy for effective management of patients with jaw fractures.

For discussion, we propose a definition of an “abbreviated” MST protocol, which is implemented without tracheostomy or gastrostomy. In the admission and triage department, with an injury severity of 1.0–2.0 points, an abbreviated MST protocol is performed without tracheostomy, after which the patient is transferred to a specialized general care department. Following the performed surgeries (under emergency and urgent protocols), the severity of the general condition was assessed using the MFS-SS scale.

By the end of the first period of TD, patients in the first group showed an improvement in their general condition to 46 ± 0.48 points [15].

Under an injury severity of 2.0–12.0 points and decompensation of the general condition, Stage 1 MST is performed in full. In other variants, an abbreviated MST protocol without tracheostomy or gastrostomy is performed. With an injury severity > 12.0 points (averaging 18.8 ± 1 point), MST is performed in full (Fig. 3).

The conducted clinical and biometric studies allowed us to develop an MST protocol, which takes into account the injury severity and the level of compensation for impaired functions. The algorithm of the developed MST is presented in Figure 3.

Stage 1 of multistage surgical treatment

In cases of the injury severity of 1.0–2.0 points and impaired body functions (MFS-SE scale score of 18.8 ± 0.9 points), under emergency indications, MST is performed using an abbreviated protocol without tracheostomy. Subsequently, patients are transferred to a specialized department where conservative management of the impaired functions is conducted during the 2nd and 3rd periods of TD. After full stabilization of the condition (MFS-SS score below 50 points), a scheduled operation to correct the anatomical changes is performed. Emergency surgeries are performed on the chest, abdomen, major vessels, pelvis, and extremities.

In cases of the injury severity of 2.0–12.0 points with decompensation of the general condition (MFS-SE scale score greater than 26.2 ± 0.8 points), an emergency operation is performed in a shock room, after which patients are transferred to an ICU.

In cases where the injury severity exceeds 12.0 points and impaired body functions (MFS-SE scale score of 42.2 ± 3.3 points) are present, Stage 1 of MST is performed in full. This includes:

- elimination of life-threatening consequences of trauma (management of asphyxia and performance of tracheostomy, establishment of conditions for conducting intensive therapy, hemostasis, relief of brain compression);
- temporary fracture fixation using orthopedic methods to achieve hemostasis;
- primary soft tissue plastic surgery elements.

These measures are carried out simultaneously in a shock room with support of specialists of other profiles. Subsequently, the patients are transferred to an ICU. Urgent surgeries are performed on the head and brain, chest, abdomen, pelvic organs, and major vessels. Delayed surgeries are also performed on the pelvic bones, spine, spinal cord, and long tubular bones.

Stage 2 of multistage surgical treatment

This stage corresponds to the 2nd and 3rd periods of TD, lasting from Day 2 to Day 10. The patients are on prolonged mechanical ventilation and receive comprehensive infusion-transfusion and antibacterial therapy (from Day 5, therapy is adjusted based on microbiological monitoring results).

Starting from Day 3, a prognosis concerning the development of severe and extremely severe disease courses is made (according to the MFS-SS scale: 50–69 points — severe form of TD; 70–98 points — extremely severe form of TD). In cases of severe TD forms with a favorable outcome and a negative prognosis for the development of infectious complications, MST is performed using an abbreviated protocol. Starting from Days 4–10, based on bacterial culture results, targeted antibiotic therapy is administered along with minimally invasive surgeries (sanitation of potential sources of infectious complications). In certain cases, staged fixation of fragments is performed using minimally invasive external osteosynthesis.

In cases of an extremely severe form of TD with an unfavorable outcome, or with a favorable outcome but a positive prognosis for the development of infectious complications (calculated using the linear discriminant function (LDF) with two formulas, see note to Fig. 3), MST is performed in full. At this stage, exhaustive diagnostics of damaged structures using computed tomography (CT) is also carried out. Creation of conditions for functional rest for the damaged structures is a component of comprehensive treatment, including the use of enteral tubes via the piriform aperture or placement of a gastrostomy. The second period concludes upon the resolution of the patient's complications and the normalization of the general condition to a level of compensation: below 50 points on the MFS-SS scale.

Bacteriological monitoring, antibacterial therapy, immunotherapy, immunocorrection, and surgical management of complications are applied.

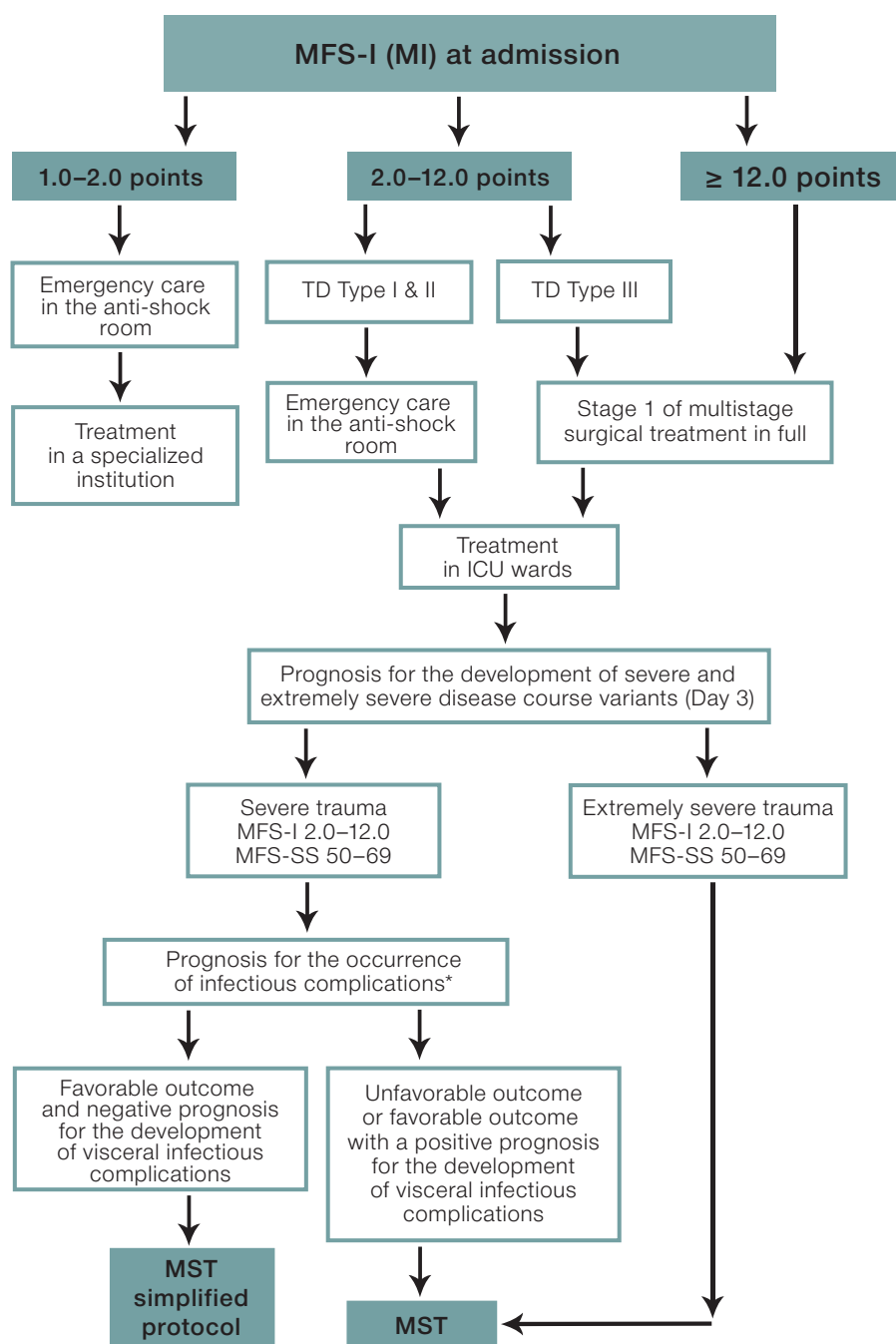


Figure prepared by the authors

Fig. 3. Algorithm of multistage surgical treatment: MFS-I — Military Field Surgery (Injury); MFS-I (MI) — Military Field Surgery (Mechanical Injury); MFS-SS — Military Field Surgery (Specialized State); TD — trauma disease; ICU — intensive care unit; MST — multistage surgical treatment

Note: * — calculation of the linear discriminant function (LDF) using formulas: $LDF_1 = 7.01X_1 + 1.34X_2 + 0.31X_3 - 10.42$ и $LDF_2 = 9.09X_1 + 1.07X_2 + 0.17X_3 - 10.24$, where X_1 — abs. lymphocyte count on Day 3, X_2 — Leukocyte Index of Intoxication (LII) on Day 3, X_3 — Injury severity according to the MFS-I (MI) scale; if $LDF_1 > LDF_2$ — positive prognosis for the development of purulent complications; if $LDF_1 < LDF_2$ — negative prognosis for the development of purulent complications.

Stage 3 of multistage surgical treatment

This reconstructive and restorative stage corresponds to the 4th period of TD. Stage 3 can be initiated only after the complete stabilization of the patient's general condition. Surgical interventions are performed in full in the specialized

departments of a Level I trauma center (neurosurgery, maxillofacial surgery, traumatology, and other departments).

When conducting a comparative analysis, our data showed good agreement with those from the Military Field Surgery Department of the Kirov Military Medical Academy [1].

CONCLUSIONS

The conducted study allowed us to achieve the research objectives. An MST methodology for combined midface injuries, which takes into account the severity of trauma and impaired body functions, has been developed. The MST strategy for patients with combined midface trauma represents a set of surgical interventions that are implemented starting from the first day of admission and are completed with reconstructive and restorative operations.

Depending on the severity of injury and the nature of the TD course, the range of surgical interventions performed within the framework of MST is differentiated. In severe TD, the likelihood of immediate outcomes and the probability of visceral infectious complications are assessed. In cases where the immediate outcome is predicted to be favorable and the prognosis for the development of visceral infectious complications is negative, then MST is carried out according to a simplified, abbreviated protocol. In cases where the prognosis for immediate outcomes is unfavorable, or when the immediate outcome is favorable but visceral infectious complications are probable, MST is performed in full.

When studying the nature of TD course in patients with craniofacial trauma, its following types were established:

- Type I — development of non-life-threatening complications and manifestation of the 2nd period, characterized by relative stabilization of impaired functions;
- Type II — development of life-threatening complications and absence of the 2nd period;
- Type III — severe trauma (polytrauma).

The implementation of the conventional protocol in 20 patients with extremely severe combined craniofacial injuries led to the development of infectious complications in 77.8% of cases, with lethality being observed in 44.4% of cases. The developed MST allowed a 47.7% reduction in the frequency of infectious complications and a 43.7% reduction in lethality to be achieved. Thus, among 22 patients with comparable injuries, infectious complications were noted in 41.6% (9 patients; $p < 0.05$), and lethality was 25% (5 patients; $p < 0.001$).

The advantage of the developed MST strategy consists in an optimal scope of diagnostic and therapeutic measures, dynamically adapted to the progression of the pathological process, alongside the development and integration of modern technologies.

References

1. Gumanenko YeK, Zavrazhnov AA, Suprun AYU, Khromov AA. Severe combined trauma and polytrauma: definition, classification, clinical characteristics, treatment outcomes. *Polytrauma*. 2021;4:6–17 (In Russ.). <https://doi.org/10.24412/1819-1495-2021-4-6-17>
2. Kharitonov DYU, Dmitriev VV, Stepanov IV, Podoprigora AV, Moiseeva VS, Garshina MA. Dependence of the severity of brain damage on the localization of facial fractures in patients with combined craniofacial injuries. *Journal of New Medical Technologies*. 2019;26(1):22–5 (In Russ.).
3. Fokas NN, Levenetc AA, Gorbach NA, Pavlushkin AA, Metelev IA, Kravtsova GN. Research methodology traumatic injuries maxillofacial (for example Krasnoyarsk Region). *International Journal of Applied and Fundamental Research*. 2015;10–5:826–9 (In Russ.). EDN: [ULVDCR](https://doi.org/10.24412/1819-1495-2021-4-6-17)
4. Maslyakov VV, Barachevsky YuE, Pavlova ON, Proshin AG, Polikarpov DN, Pimenov AV, et al. Analysis of the results of treatment of combined and isolated injuries of the facial skull resulting from traffic accidents in regional trauma centers. *Polytrauma*. 2021;4:28–38 (In Russ.). <https://doi.org/10.24412/1819-1495-2021-4-28-37>
5. Belen'kiy IG, Manukovskii VA, Tulupov AN, Demko AE, Kandyba DV, Sergeev GD, et al. Strategies of Osteosynthesis: Problems and Perspectives. *Traumatology and Orthopedics of Russia*. 2022;28(2):79–90 (In Russ.). <https://doi.org/10.17816/2311-2905-1693>
6. Grebnev GA, Sviridenko AD, Krasikov AV. Comparative characteristics of classifications of injuries of the middle zone of the face. *Russian Journal of Dentistry*. 2021;25(4):307–13 (In Russ.). <https://doi.org/10.17816/1728-2802-2021-25-4-307-313>
7. Pfeifer R, Kalbas Y, Pape H-C. The concept of “Damage control” in polytrauma: what are standards in 2021? *Polytrauma*. 2021;2:10–8 (In Russ.). <https://doi.org/10.24412/1819-1495-2021-2-10-18>
8. Pfeifer R, Kalbas Y, Coimbra R, Leenen L, Komadina R, Hildebrand F, et al. Indications and interventions of damage control orthopedic surgeries: an expert opinion survey. *European Journal of Trauma Emergency Surgery*. 2020;47:2081–92. <https://doi.org/10.1007/s00068-020-01386-1>
9. Abu-Zidan FM, Jawas A, Idris K, Cevik AA. Surgical and Critical Care Management of Earthquake Musculoskeletal Injuries and Crush Syndrome: A Collective Review. *Turkish Journal of Emergency Medicine*. 2024;24(2):67–79. https://doi.org/10.4103/tjem.tjem_11_24
10. Arnaouti MKC, Cahill G, Baird MD, Mangurat L, Harris R, Edme LPP, et al. Medical Disaster Response: A Critical Analysis of the 2010 Haiti Earthquake. *Frontiers in Public Health*. 2022;1(10):9955–95. <https://doi.org/10.3389/fpubh.2022.995595>
11. Tribble DR, Murray CK, Lloyd BA, Ganesan A, Mende K, Blyth DM, et al. After the Battlefield: Infectious Complications among Wounded Warriors in the Trauma Infectious Disease Outcomes Study. *Military Medicine*. 2019;184:18–25. <https://doi.org/10.1093/milmed/usz027>
12. Stewart L, Li P, Blyth DM, Campbell WR, Petfield JL, Krauss M, et al. Antibiotic practice patterns for extremity wound infections among blast-injured subjects. *Military Medicine*. 2020;185:628–36. <https://doi.org/10.1093/milmed/usz211>
13. McDonald J, Liang SY, Li P, Stewart L, Tribble DR. DoD-VA trauma infection research collaboration. *Military Medicine*. 2022;187:17–24. <https://doi.org/10.1093/milmed/usab508>
14. Trajano AD, Pereira BM, Fraga GP. Epidemiology of in-hospital trauma deaths in a Brazilian university hospital. *BMC*

Emergency Medicine. 2014;14:22.

<https://doi.org/10.1186/1471-227X-14-22>

15. Roberts DJ, Bobrovitz N, Zygun DA, Kirkpatrick AW, Ball CG, Faris PD, et al. Evidence for use of damage control surgery

and damage control interventions in civilian trauma patients: a systematic review. *World Journal of Emergency Surgery*. 2021;16(1):10.

<https://doi.org/10.1186/s13017-021-00352-5>

Authors' contributions. All authors confirm that their authorship complies with the ICMJE criteria. The primary contributions are distributed as follows: Yurii A. Shcherbuk — responsibility for the management and coordination of research planning and execution; Olga D. Madai — data visualization, material processing, analysis of literary data, manuscript writing; Dmitrii Yu. Madai — article concept, design and conception of the clinical case, responsibility for the integrity of all parts of the article, text editing, final approval of the manuscript, final manuscript formatting, and responsibility for the management and coordination of research planning and execution; Alexandr Yu. Shcherbuk — draft editing.

AUTHORS

Yurii A. Shcherbuk, Dr. Sci. (Med.), Academician of RAS, Professor

<https://orcid.org/0000-0003-1945-6959>
9361661@gmail.com

Olga D. Madai, Cand. Sci. (Med.), Associate Professor

<https://orcid.org/0000-0003-3000-7273>
madayolga@mail.ru

Dmitrii Yu. Madai, Dr. Sci. (Med.), Professor

<https://orcid.org/0000-0002-6841-0730>
wpxmdy@mail.ru

Alexandr Yu. Shcherbuk, Dr. Sci. (Med.), Professor

<https://orcid.org/0000-0003-3049-1552>
gkod@gmail.com

<https://doi.org/10.47183/mes.2025-359>

OPTIMIZATION AND VALIDATION OF *BACILLUS SUBTILIS* SPORE INACTIVATION REGIMES IN SUPERCRITICAL CARBON DIOXIDE: PURE GAS, HYDROGEN PEROXIDE, PERACETIC ACID

Ramaz Sh. Gvetadze, Mariam S. Galstyan, Yaser N. Kharakh[✉], Petr Yu. Kolesnikov, Levon G. Kirakosyan, Mikhail S. Podporin, Viktor N. Tsarev, Sergey D. Arutyunov

Russian University of Medicine, Moscow, Russia

Introduction. Healthcare-associated infections (HAIs) continue to pose a significant challenge to public health. The existing sterilization methods possess a number of limitations regarding their applicability to heat-sensitive materials and the possibility of residual toxicity or material damage. Sterilization using supercritical carbon dioxide, particularly in combination with oxidative additives, appears to be a promising alternative capable of providing the required level of microbial safety at low temperatures and moderate pressures. However, the available data show significant variability depending on experimental conditions, indicating the need for optimization and validation of processing regimes.

Objective. Optimization and validation of *Bacillus subtilis* spore sterilization regimes in an experiment using supercritical carbon dioxide (scCO₂), including pure scCO₂, scCO₂ with hydrogen peroxide (H₂O₂), and scCO₂ with peracetic acid (PAA).

Materials and methods. Experiments were conducted using a prototype test bench for investigating sterilization regimes. *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 NCTC 10400 was used as the biological indicator. The study involved the contamination of titanium discs with spores followed by their treatment under the three regimes (pure scCO₂, scCO₂ + H₂O₂, scCO₂ + PAA). Further, microbiological analysis with enumeration of surviving spores and statistical processing of the results were carried out. For each regime, central composite experimental designs were implemented, varying temperature, pressure, and exposure duration. Efficacy was assessed based on the log-reduction of viable spores. Validation series included 10 independent replicates each.

Results. Gas-dynamic treatment with pure scCO₂ in the range of 35–60°C, 70–120 atm, and 60–120 min proved ineffective (maximum log-reduction ≤ 0.8). The addition of oxidizing agents significantly increased the degree of inactivation: for the scCO₂ + H₂O₂ (200 ppm) regime, the optimal parameters were 37.9°C, 120 atm, 30 min (log-reduction 4.4 ± 0.3; CV = 7.3%); for scCO₂ + PAA (50 ppm) — 45°C, 94 atm, 10 min (log-reduction 6.0 ± 0.3; CV = 4.9%), which meets the requirements for the sterility assurance level (SAL) for medical devices. The PAA regime provided a statistically significant higher efficacy compared to H₂O₂ (*t*-test, *p* < 0.001).

Conclusions. The sterilization methodology based on supercritical carbon dioxide with the addition of hydrogen peroxide or peracetic acid ensures effective inactivation of *Bacillus subtilis* spores under parameters compatible with the low-temperature processing of heat-sensitive medical materials, thus meeting modern sterility requirements for medical devices.

Keywords: *Bacillus subtilis*; sterilization of thermolabile medical devices; carbon dioxide sterilization; peracetic acid sterilization; hydrogen peroxide sterilization; sterilization; gas sterilization

For citation: Gvetadze R.Sh., Galstyan M.S., Kharakh Ya.N., Kolesnikov P.Yu., Kirakosyan L.G., Podporin M.S., Tsarev V.N., Arutyunov S.D. Optimization and validation of *Bacillus subtilis* spore inactivation regimes in supercritical carbon dioxide: Pure gas, hydrogen peroxide, peracetic acid. *Extreme Medicine*. 2026;28(1):134–145. <https://doi.org/10.47183/mes.2025-359>

Funding: the study was carried out within the framework of the scientific research support program of the Russian University of Medicine (Ministry of Health of Russia) (NPO-0014-N).

Compliance with the ethical principles: an Ethics Committee approval was not required since the study was conducted *in vitro* without involving humans, animals, clinical specimens, or personal data.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Yaser N. Kharakh c.kharakh@gmail.com

Received: 22 July 2025 **Revised:** 22 Sep. 2025 **Accepted:** 16 Oct. 2025 **Online first:** 30 Oct. 2025

УДК 579.832/833:615.47

ОПТИМИЗАЦИЯ И ВАЛИДАЦИЯ РЕЖИМОВ ИНАКТИВАЦИИ СПОР *BACILLUS SUBTILIS* В СРЕДЕ СВЕРХКРИТИЧЕСКОГО CO₂: ЧИСТЫЙ ГАЗ, ПЕРЕКИСЬ ВОДОРОДА, НАДУКСУСНАЯ КИСЛОТА

Р.Ш. Гветадзе, М.С. Галстян, Я.Н. Харах[✉], П.Ю. Колесников, Л.Г. Киракосян, М.С. Подпорин, В.Н. Царев, С.Д. Арутюнов

Российский университет медицины, Москва, Россия

Введение. Инфекции, связанные с оказанием медицинской помощи (ИСМП), продолжают представлять существенную проблему для здравоохранения. Существующие методы стерилизации имеют ограничения по применимости к термочувствительным материалам и могут сопровождаться остаточной токсичностью или повреждением изделий. Стерилизация сверхкритическим диоксидом углерода, особенно в сочетании с окислительными добавками, представляется перспективной альтернативой, способной обеспечить требуемый уровень микробной безопасности при низких температурах и умеренных давлениях. Однако существующие данные демонстрируют значительную вариабельность результатов в зависимости от условий эксперимента, что указывает на необходимость оптимизации и валидации режимов обработки.

Цель. Оптимизация и валидация режимов стерилизации спор *Bacillus subtilis* в эксперименте с использованием сверхкритического диоксида углерода (scCO₂), включая чистый scCO₂, scCO₂ с перекисью водорода (H₂O₂) и scCO₂ с надуксусной кислотой (PAA).

© R.Sh. Gvetadze, M.S. Galstyan, Ya.N. Kharakh, P.Yu. Kolesnikov, L.G. Kirakosyan, M.S. Podporin, V.N. Tsarev, S.D. Arutyunov, 2025

Материалы и методы. Эксперименты проводили с использованием прототипа испытательного стенда для исследования режимов стерилизации. В качестве биологического индикатора использовали *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 NCTC 10400. Исследование включало контаминацию титановых дисков спорами; обработку тремя режимами (чистый scCO_2 , $\text{scCO}_2 + \text{H}_2\text{O}_2$, $\text{scCO}_2 + \text{PAA}$); микробиологический анализ с подсчетом выживших спор; статистическую обработку результатов. Для каждого режима были реализованы центральные композиционные планы экспериментов с варьированием температуры, давления и времени экспозиции. Эффективность оценивали по показателю log-reduction жизнеспособных спор, валидационные серии включали по 10 независимых повторов.

Результаты. Газодинамическая обработка чистым scCO_2 в диапазоне 35–60 °C, 70–120 атм, 60–120 мин оказалась неэффективной (максимальный log-reduction $\leq 0,8$). Добавление окислителей существенно увеличивало степень инактивации: для режима $\text{scCO}_2 + \text{H}_2\text{O}_2$ (200 ppm) оптимальные параметры составили 37,9 °C, 120 атм, 30 мин (log-reduction $4,4 \pm 0,3$; CV = 7,3%); для $\text{scCO}_2 + \text{PAA}$ (50 ppm) — 45 °C, 94 атм, 10 мин (log-reduction $6,0 \pm 0,3$; CV = 4,9%), что соответствует требованиям уровня гарантии стерильности (SAL) для медицинских изделий. Режим с PAA обеспечил статистически значимо более высокую эффективность по сравнению с H_2O_2 (*t*-тест, $p < 0,001$).

Выводы. Методика стерилизации на основе сверхкритического диоксида углерода с добавлением перекиси водорода или надуксусной кислоты обеспечивает эффективную инактивацию спор *Bacillus subtilis* при параметрах, соответствующих низкотемпературной обработке термочувствительных медицинских материалов, и удовлетворяет современным требованиям к стерильности медицинских изделий.

Ключевые слова: *Bacillus subtilis*; стерилизация термолабильных медицинских изделий; стерилизация диоксидом углерода; стерилизация надуксусной кислотой; стерилизация перекисью водорода; стерилизация; газовая стерилизация

Для цитирования: Гветадзе Р.Ш., Галстян М.С., Харах Я.Н., Колесников П.Ю., Киракосян Л.Г., Подпорин М.С., Царев В.Н., Арутюнов С.Д. Оптимизация и валидация режимов инактивации спор *Bacillus subtilis* в среде сверхкритического CO_2 : чистый газ, перекись водорода, надуксусная кислота. *Медицина экстремальных ситуаций*. 2026;28(1):134–145. <https://doi.org/10.47183/mes.2025-359>

Финансирование: исследование выполнено в рамках программы поддержки научных исследований ФГБОУ ВО «Российский университет медицины» Минздрава России (НПО-0014-Н).

Соответствие принципам этики: одобрение биоэтического комитета не требовалось, поскольку исследование выполнено *in vitro* без участия людей, животных, клинических образцов и использования персональных данных.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Харах Ясер Насерович c.kharakh@gmail.com

Статья поступила: 22.07.2025 **После доработки:** 22.09.2025 **Принята к публикации:** 16.10.2025 **Online first:** 30.10.2025

INTRODUCTION

Healthcare-associated infections (HAIs) continue to pose a significant challenge to public health. According to a number of publications, the surfaces of medical devices can facilitate the formation of biofilms and the spread of resistant pathogens, complicating the prevention and treatment of HAIs [1–3]. Several studies have noted the role of tightening requirements for device safety and biocompatibility in the adoption of new materials and more complex designs, which may limit the applicability of some conventional sterilization methods due to potential risks of degradation or residual toxicity [2, 4]. Well-established sterilization methods, such as autoclaving, radiation, and gas treatment (e.g., with ethylene oxide), have limited applicability to heat-sensitive materials due to the possibility of residual toxicity or structural alterations in devices [4]. In this context, sterilization using supercritical carbon dioxide (scCO_2) is considered a promising alternative as a method integrating efficacy, safety, and compatibility with modern materials.

In order to reduce the risk of device contamination, the inactivation of both vegetative and spore-forming bacteria should be ensured. Among spore-forming species, *Bacillus subtilis* is widely used as a model test organism in sterilization studies [5–7]. Given the increasing constraints on materials and device designs, there is a growing interest in novel low-temperature methods that exhibit effectiveness while preserving the functional properties of materials. Spore-forming bacilli, particularly

B. subtilis and *B. stearothermophilus*, are known to be among the most resistant microorganisms to eradication due to the protective structural features of their spores, making them benchmark indicators of sterilization efficacy [5, 7].

A number of experimental studies have demonstrated that the use of scCO_2 , as well as its combinations with various oxidizing agents, can effectively inactivate *B. subtilis* spores under different processing parameters [5–9]. The authors of most publications emphasize that the proposed sterilization regimes cannot be considered a versatile solution; moreover, the existing data show a significant variability in effectiveness depending on experimental conditions and protocols used [5–7, 10]. Therefore, in our study, the ranges of parameters are selected following a review of literature data and considering the engineering constraints of our laboratory facility (maximum pressure 120 atm).

In this work, we aim to optimize and validate sterilization regimes for *Bacillus subtilis* spores in an experiment using supercritical carbon dioxide (scCO_2), including pure scCO_2 , scCO_2 with hydrogen peroxide, and scCO_2 with peracetic acid.

MATERIALS AND METHODS

Experimental facility

The experiments were conducted using a prototype test bench for studying sterilization regimes (Skolkovo

Institute of Science and Technology, Russia). The facility included a 1 L working chamber made of AISI 316 stainless steel, with temperature control from ambient to 100°C and a maximum working pressure of 120 atm. The system was equipped with a programmable logic controller (PLC) for automatic cycle control, redundant pressure monitoring (analog pressure gauges and digital sensors), and a PID temperature controller (TPM210). Medical-grade CO₂ was used as the sterilizing agent. Figure 1 presents a general schematic description of this facility.

Biological Indicator

The microorganism *Bacillus subtilis* subsp. *Spizizenii* ATCC 6633 NCTC 10400 was used as the biological indicator.

Culture preparation and sample contamination

Nutrient agar medium No. 1 GRM of the following composition was used for cultivation: casein hydrolysate (5.00 g/L); yeast extract (2.50 g/L); glucose (1.00 g/L); and agar-agar (9.00 g/L). Inoculations were incubated at a temperature of 37.1°C for 24 h.

The inoculum was prepared from a 24-h agar culture by selecting well-isolated colonies and transferring them into a sterile 0.9% sodium chloride (NaCl) solution to a concentration equivalent to 0.5 McFarland standard units [11]. The suspension density was controlled using a DEN-1 densitometer (Biosan, Latvia). The inoculum was used within 15 min of preparation.

A volume of 100 µL of the inoculum was applied onto the surface of titanium discs using an automatic micropipette and dried to completeness at a temperature of 20 ± 2°C under sterile conditions. The contaminated

discs were packaged in individual kraft paper bags prior to gas-dynamic treatment (Fig. 2A).

Microbiological analysis

Following sterilization, the samples were removed from the kraft bags and placed into sterile containers with 5 mL of 0.9% sodium chloride (NaCl) solution. The wash-off was performed by shaking for 1.5 min at 1000 rpm using a V-3 vortex mixer.

The wash fluid was plated in a volume of 200 µL (for screening experiments) or 1000 µL (for validation experiments with SAL calculation) onto the surface of nutrient agar in 90 mm Petri dishes. The difference in plating volumes is related to methodological requirements. Thus, for screening experiments, a 200 µL volume provided sufficient sensitivity while reducing the load on the automatic counting system Scan 500; for validation experiments, the 1000 µL volume maximized the sensitivity of the analysis in accordance with GOST R ISO¹. The fluid was evenly distributed over the surface of the medium using a sterile Drigalski spatula. The inoculations were incubated at 37°C for 24 h.

The results were recorded both visually and using a Scan 500 automatic colony counter (Interscience, France). For plates with confluent growth, counting was performed automatically using a Scan 500 system via the selected field method. The latter consisted in defining the counting area followed by calculation of the colony density and its automatic recalculation for the entire plate area, converted to CFU/mL while accounting for the inoculation volume. Reference samples were not subjected to sterilization; the wash-off from these controls was performed immediately after contamination using the same method. The minimum detectable number of viable microorganisms (limit of detection, LOD) was 1 CFU/mL.

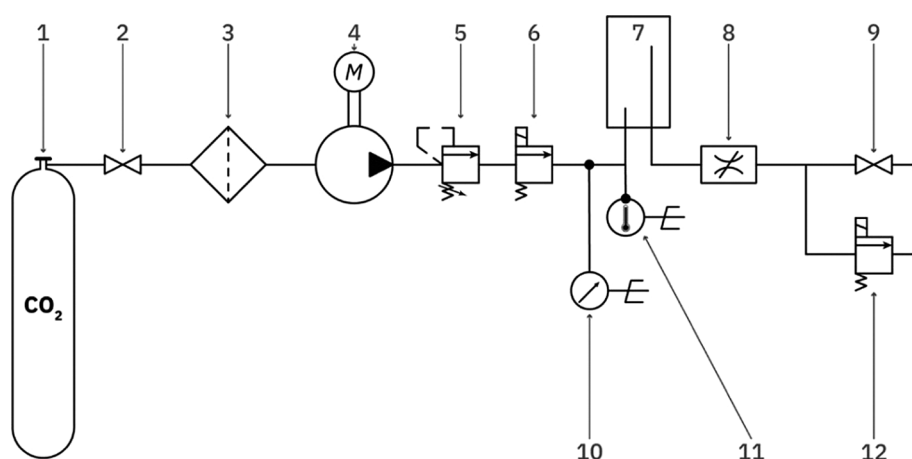


Figure prepared by the authors based on their own data

Fig. 1. Pneumatic schematic of the prototype test bench for investigating sterilization regimes: 1 — gas cylinder; 2 — first valve; 3 — filter; 4 — pump; 5 — safety valve; 6 — first control valve; 7 — working chamber; 8 — throttle valve; 9 — second control valve; 10 — electronic pressure gauge; 11 — temperature sensor; 12 — second valve

¹ GOST R ISO 14937-2012 (ISO 14937:2009). Sterilization of health care products. General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. Moscow: Standartinform; 2013.

Preparation of chemical additives

Solutions of hydrogen peroxide (H_2O_2) and peracetic acid ($\text{C}_2\text{H}_4\text{O}_3$) were prepared under controlled conditions as part of a scientific collaboration with the Mendelev Russian University of Chemical Technology. The solutions were prepared using Type I ultrapure water, obtained through double reverse osmosis and UV treatment. All preparation and dispensing procedures were carried out in a glove box under inert atmosphere.

The working concentrations were 200 ppm (0.02%) for hydrogen peroxide and 50 ppm (0.005%) for peracetic acid.

The solutions were prepared in batches, considering a shelf life of up to one week under storage conditions recommended by the Mendelev University (at low temperature, protected from light). The incoming quality control of the stock solutions was performed using redox titration methods to verify their concentration.

Experimental design

Using the Minitab 17.1 statistical software, three separate Central Composite Designs (CCD) were generated, one for each treatment group: CD (pure carbon dioxide), CDHP (carbon dioxide + hydrogen peroxide), and CDPAA (carbon dioxide + peracetic acid).

For the CD group, temperature (35–60°C), pressure (70–120 atm), and exposure duration (60–120 min) were varied within a single block, based on literature data [5, 7, 8] and considering the engineering constraints of the equipment ($P_{\text{max}} = 120$ atm).

The CDHP and CDPAA groups were investigated at temperatures of 30–45°C, pressures of 90–110 atm, and exposure duration of 10–30 min, according to

data from [9, 12], with the experiments organized into three blocks.

Each Central Composite Design (CCD) included 20 experimental runs: 8 cube points, 6 axial points, and center points with $\alpha = 1$. All equipment operations were performed by a single operator, while all microbiological procedures were conducted by one microbiologist to ensure data homogeneity.

The validation of optimal sterilization regimes involved conducting a series of 10 independent replicates for each regime, followed by the calculation of the log-reduction relative to the control (N_0). When more than two models and corresponding optimal regimes were identified, a comparative analysis of the log-reduction values between the groups was planned for the statistical assessment of differences in sterilization efficacy.

The experimental runs were performed according to the CCD matrix, with process parameter deviations controlled within $\pm 2^\circ\text{C}$ for temperature, ± 5 atm for pressure, and ± 2 min for exposure time.

Experimental procedure

Each experiment involved the preparation of two samples (on a matrix of titanium discs measuring 8×1 mm): a reference and a test (experimental) sample. The test samples for the CD regime were packaged in kraft paper (Fig. 2A). For the CDHP and CDPAA regimes, they were additionally fully immersed in 15 mL of the corresponding additive solution in open test tubes placed inside the reaction chamber (Fig. 2B).

The experimental procedure involved sample preparation and loading into the working chamber, followed by sealing and achieving the specified CO_2 temperature and pressure conditions. After maintaining the target

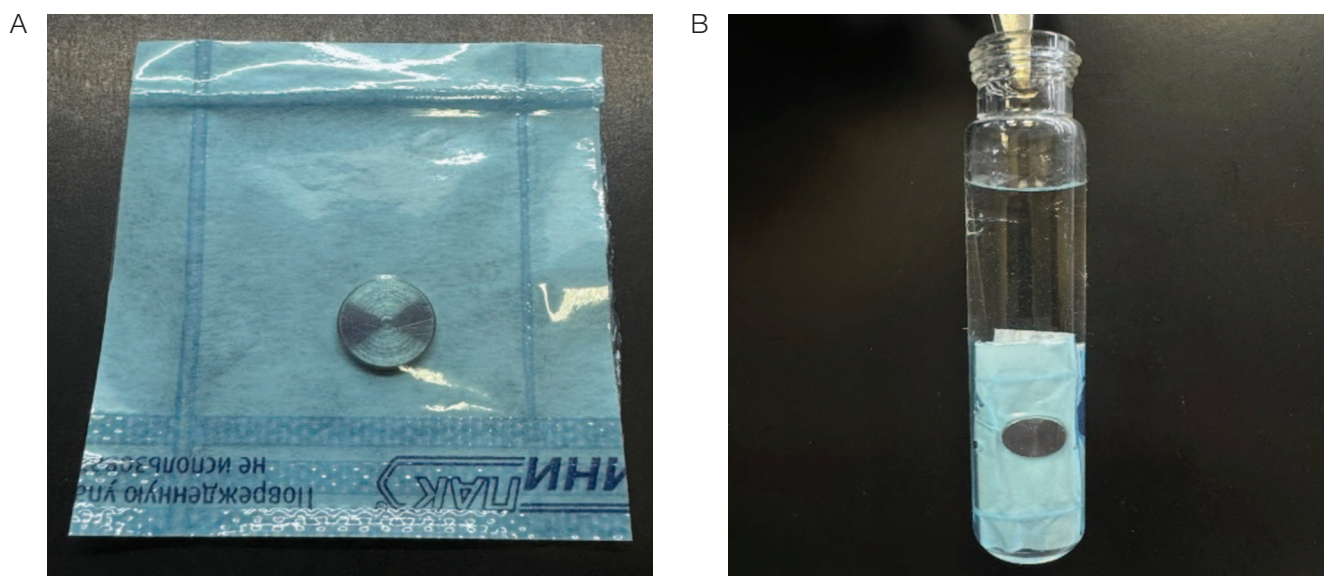


Figure prepared by the authors based on their own data

Fig. 2. Preparation of titanium discs for gas-dynamic treatment: A — individual packaging of a contaminated titanium disc in a kraft paper bag; B — placement of the packaged disc in a test tube with oxidizer solution for treatment according to CDHP or CDPAA protocols

conditions for the designated exposure time, a controlled decompression (≤ 10 atm/min to minimize mechanical inactivation effects) was performed, followed by sample retrieval and microbiological analysis.

The process parameters (temperature, pressure, duration) were monitored manually using redundant analog and digital sensors. Experiments were conducted at an ambient temperature of 20–25°C and a relative humidity of $50 \pm 10\%$.

Statistical analysis

The logarithmic reduction (log-reduction), calculated using formula (1), was used as the response variable:

$$\log - \text{reduction} = \log_{10} \left(\frac{N_0}{N} \right), \quad (1)$$

where N_0 — the number of viable microorganisms in the reference sample;

N — after treatment.

To identify the dependence of sterilization efficacy on process parameters, the response surface methodology (RSM) with the construction of full quadratic models was applied.

For each experimental group, a preliminary assessment of data distribution using the Anderson–Darling (AD) test was planned. Depending on the outcome of the normality test, either a two-sample t -test (in the absence of significant deviations) or a non-parametric Mann–Whitney U test (MW), corrected for ties, was planned for comparing reference and test samples. The sterility of medical devices was considered achieved at a log-reduction ≥ 6 , which corresponds to a Sterility Assurance Level (SAL) of $\leq 10^{-6}$ according to GOST requirements².

RESULTS

Results for the CD Group

Raw data and response distribution. In the experiment on the treatment of *Bacillus subtilis* spores with pure CO_2 , 20 runs were conducted, varying temperature (35–60°C), pressure (70–120 atm), and exposure duration (60–120 min). The mean log-reduction was 0.52 ± 0.35 (AD: $p > 0.05$).

DOE Analysis: Full Quadratic Model. An analysis of variance (ANOVA) indicated the lack of statistical significance for the model ($F = 0.26$; $p = 0.972$; $R^2 = 19.1\%$, $\text{adj } R^2 = 0\%$). Only 19% of the variability can be explained by the process factors (temperature, pressure, exposure duration), while the remaining 81% is attributed to random error. None of the coefficients, including main, quadratic, and interaction terms, were statistically significant (all $p > 0.2$).

With the purpose of verifying the robustness of the conclusion regarding the statistical insignificance of the

full quadratic model, a stepwise variable elimination procedure (backward elimination method) was additionally applied at $\alpha = [0.1; 0.3]$ (step = 0.1). In all cases, the final model was reduced to a constant, since no variable reached statistical significance (minimum $p = 0.24$), and the explained variance did not exceed 10%.

Statistical analysis of differences between groups

In the control group CD (N_0), the median number of viable spores was 4.34×10^3 [2940; 5245] CFU/mL, compared to 1.57×10^3 [556; 2678] CFU/mL in the treated CD group (N).

A test for normality of the data distribution showed no deviations from a normal distribution in the treated group (AD: $p > 0.05$). However, a statistically significant deviation was detected in the control group (AD: $p < 0.05$). Consequently, a non-parametric test was applied for intergroup comparison, which revealed a statistically significant difference (MW: $p < 0.001$). Thus, the reduction in the number of viable spores after treatment with pure CO_2 was established to be statistically significant.

Results for the CDHP group

Raw data and the dynamics of experimental series expansion. In the initial series of experiments on treating *Bacillus subtilis* spores with a mixture of CO_2 and hydrogen peroxide (CDHP), 20 runs were performed, varying temperature (30–45°C), pressure (90–110 atm), and exposure duration (10–30 min). An analysis of the constructed regression model revealed a low explained variance ($R^2 = 45\%$; $\text{adj } R^2 = 0\%$). All coefficients, including linear, quadratic, and interaction effects, were statistically insignificant ($p > 0.4$), and the lack-of-fit test showed a high p -value ($p = 0.94$), indicating the inability of the model to explain the variation in the experimental data. A stepwise factor elimination procedure (backward elimination method) failed to select any predictors, and the final model included only a constant.

Given that the structure of the initial experimental design was unbalanced—the majority of replicates were concentrated at the center point (seven replicates), while only two experiments were performed at the extreme factor levels (pressure of 90 and 110 atm; exposure duration of 10 and 30 min), and independent replicates were missing for several factor combinations — these results collectively indicated insufficient informativeness of the initial design and justified the need for targeted expansion of the series.

Consequently, five points were added, located in the central region and at the boundaries of the varying factors (Table 3, No. 21–25). In the expanded sample ($n = 25$), a slight improvement in model parameters was observed ($R^2 = 40\%$; $\text{adj } R^2 = 4\%$); however, key effects remained insignificant ($p > 0.25$ for all factors; lack-of-fit:

² GOST R ISO 14937-2012 (ISO 14937:2009). Sterilization of health care products. General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. Moscow: Standartinform; 2013.

$p = 0.97$), and the stepwise elimination procedure again failed to identify statistically significant predictors. To further investigate the influence of pressure on sterilization efficacy, six points with extreme pressure values (70 and 120 atm, at a fixed temperature of 38°C) were added. The median log-reduction for the full sample ($n = 31$) was 1.31 [0.50; 2.61] (AD: $p < 0.05$).

Final analysis of the experimental series ($n = 31$): Model and statistical parameters

In order to determine the influence of factors on spore inactivation efficacy, a multiple regression model was constructed using a stepwise backward elimination procedure ($\alpha = 0.1$). The final model included the linear and quadratic effects of temperature and pressure, as well as the linear effect of exposure duration. Interaction effects between factors were excluded as non-significant. The regression equation for the predicted log-reduction value for the CDHP group (Y_{CDHP}) is as follows:

$$Y_{CDHP} = 1.60 + 0.387 \times t_C - 0.1800 \times P_{atm} + 0.01542 \times T_{min} - 0.00511 \times t_C^2 + 0.000996 \times P_{atm}^2. \quad (2)$$

The explanatory power of the model increased to 58.5% ($R^2 = 58.5\%$; $adj R^2 = 50.2\%$; $pred R^2 = 38.5\%$; lack-of-fit: $p = 0.32$). The quadratic terms for temperature ($p < 0.05$) and pressure ($p < 0.001$), as well as the linear effect of pressure ($p < 0.05$), were found to be statistically significant, while all other variables were excluded during the selection process due to a lack of significance ($p > 0.1$).

Statistical analysis of differences between groups

In the CDHP control group (N_0 , $n = 31$), the median number of viable spores was 3.1×10^3 [2950; 3680] CFU/mL, compared to 1.9×10^2 [101; 474] CFU/mL in the treated group (N , $n = 31$). Testing for normality in the

distribution of CFU/mL revealed statistically significant deviations from a normal distribution in both groups (AD: $p < 0.005$). In this light, a non-parametric test was applied for intergroup comparison, the results of which showed a statistically significant difference between the groups (MW: $p < 0.0001$). The treatment effect is clearly demonstrated by the appearance of the reference and treated samples (Fig. 3).

Search for optimal processing conditions

In order to determine optimal sterilization regimes, an analysis of the obtained regression model (Y_{CDHP}) was performed. The maximum value of the log-reduction within the range of the investigated factors was used as the optimality criterion (Fig. 4).

The calculation results were used to determine that the maximum effect is achieved at a temperature of 37.9°C, a pressure of 120 atm, and an exposure duration of 30 min (Fig. 5). Under these conditions, the predicted log-reduction value is 2.14 (95% CI: [1.78; 2.50]; 95% PI (prediction interval): [1.42; 2.87]), and the integral optimality criterion (D) for the identified solution is 0.78.

Model diagnostics

Diagnostics of the final regression model ($n = 31$) was performed based on visual analysis of residual distribution. The normal probability plot (Fig. 6A) revealed a deviation of residuals from the normal distribution; however, no explicit outliers or structural patterns were detected. Similarly, the plot of residuals versus predicted values (Fig. 6B) showed no systematic trends or signs of heteroscedasticity.

Results for the CDPA group

Original data and response distribution. In the experiment on the treatment of *B. subtilis* spores with a

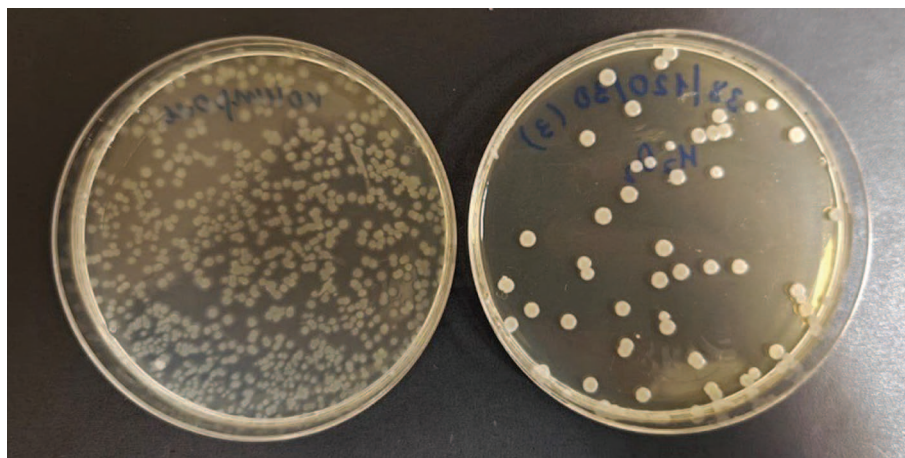


Figure prepared by the authors based on original data

Fig. 3. Example of Petri dish appearance: reference sample (left) and sample after treatment using one of the studied CDHP regimes (right)

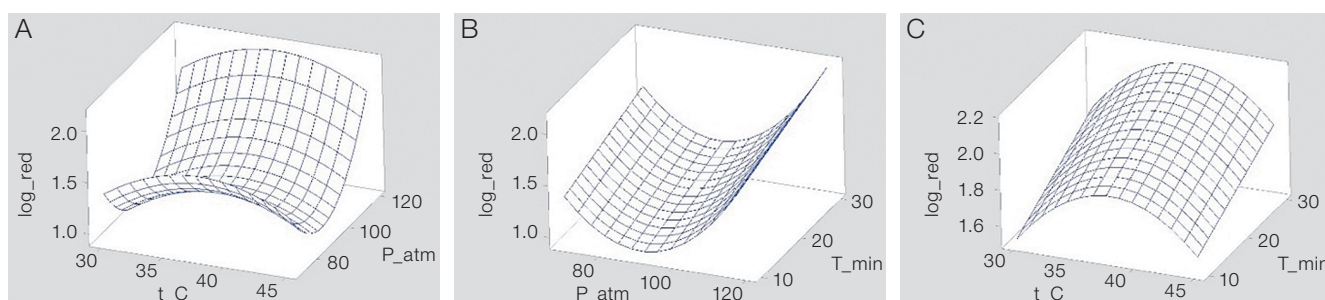


Figure prepared by the authors based on original data

Fig. 4. Response surfaces of the log-reduction parameter based on the regression model for the CDHP group ($n = 31$): A — P_{atm} and t_C at fixed T (30 min); B — P_{atm} and T_{min} at fixed t (37.9°C); C — t_C and T_{min} at fixed P (120 atm); t_C — temperature; P_{atm} — pressure, atm; T_{min} — exposure time, min; log_{red} — response (log_{10} -reduction, log_{10} units)

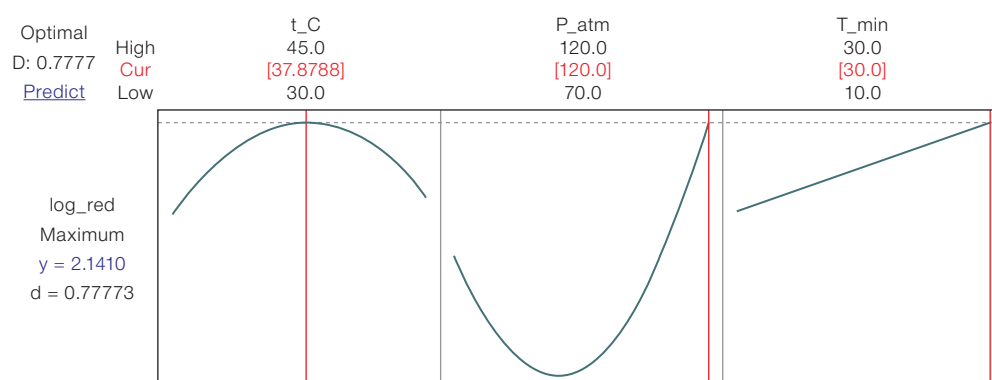


Figure prepared by the authors based on original data

Fig. 5. Graph of the search for optimal processing conditions for the CDHP group: $n = 31$; t_C — temperature, °C; P_{atm} — pressure, atm; T_{min} — exposure time, min; log_{red} — log_{10} reduction; D — composite desirability; vertical lines — optimal factor levels

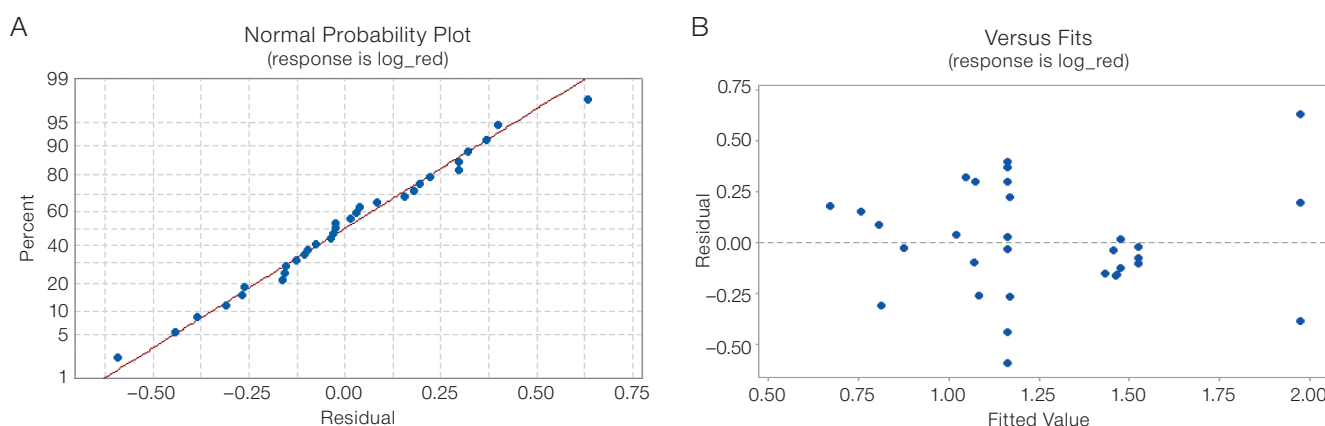


Figure prepared by the authors based on original data

Fig. 6. Diagnostics of the log-reduction model residuals (CDHP group, $n = 31$): A — normal probability plot (X-axis: residuals, log_{10} units; Y-axis: percentage, %); B — residuals vs. predicted values plot (X-axis: predicted log_{10} reduction values; Y-axis: residuals, log_{10} units)

mixture of CO_2 and peracetic acid (CDPAA), 20 runs were conducted with variations in temperature (30–45°C), pressure (90–110 atm), and exposure duration (10–30 min). The mean log-reduction was 2.10 ± 0.90 (AD: $p > 0.05$).

DOE analysis: Full quadratic model

Unlike previous experimental series, it was decided for the CDPAA regime not to simplify the full quadratic model, since its quality metrics were deemed satisfactory

($R^2 = 78.0\%$; $adj R^2 = 47.6\%$; lack-of-fit: $p = 0.08$). The regression equation for the predicted log-reduction value for the CDPAA group (Y_{CDPAA}) takes the following form:

$$Y_{CDPAA} = -19,80 + 0,163 \times t_C + 0,387 \times P_{atm} - 0,106 \times T_{min} - 0,00219 \times t_C^2 - 0,00263 \times P_{atm}^2 + 0,00027 \times t_{min}^2 + 0,00190 \times t_C \times P_{atm} - 0,00347 \times t_C \times T_{min} + 0,00207 \times P_{atm} \times T_{min}. \quad (3)$$

Of all the factors included in the model, only the linear effect of temperature was statistically significant ($p < 0.05$), whereas the effects of pressure ($p > 0.05$), exposure duration ($p > 0.05$), and the quadratic and interaction terms did not reach statistical significance (all $p > 0.22$).

Statistical analysis of intergroup differences

In the CDPAA control group (N_0 , $n = 20$), the median number of viable spores was 2.13×10^3 [1600; 3973] CFU/mL, compared to 2.21×10^1 [12.5; 41.1] CFU/mL (AD: $p < 0.005$) in the experimental group (N , $n = 20$). Statistical analysis of significance of differences

confirmed a significant reduction in viable spores after treatment (MW: $p < 0.0001$). Figure 7 shows a comparison of the appearance of the reference and test samples.

Optimization of treatment conditions

The full quadratic model Y_{CDPAA} (Fig. 8) was used to determine optimal sterilization regimes based on the criterion of log-reduction maximization.

According to the optimization results, the highest predicted log-reduction value is 3.60 (95% CI: [2.37; 4.82]; 95% PI: [1.65; 5.54]), achieved at a temperature of 45°C, pressure of 94 atm, and exposure duration of 10 min. For these conditions, the composite desirability of the model equals 0.95 (D), indicating a high degree of conformity between the selected regime and the specified parameters (Fig. 9).

Model diagnostics

Evaluation of the normal probability plot of residuals (Fig. 10A) and the plot of residuals versus predicted

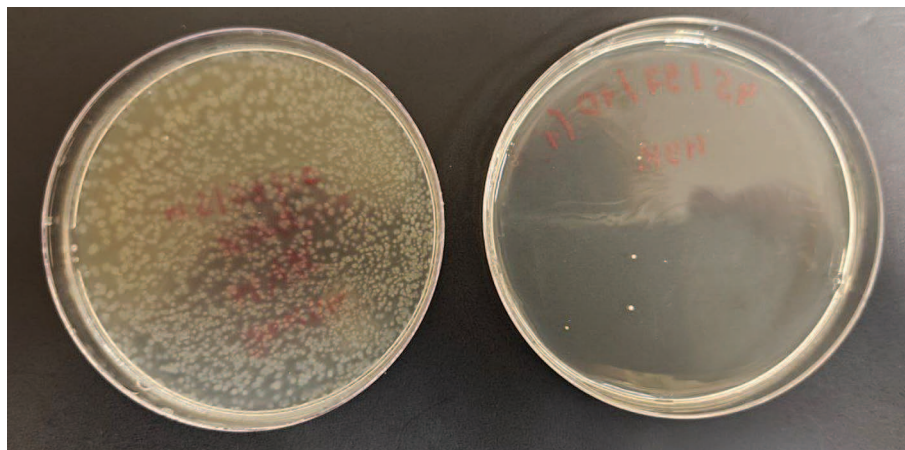


Figure prepared by the authors based on original data

Fig. 7. Example of Petri dish appearance: reference sample (left) and sample after treatment with one of the studied CDPAA regimes (right)

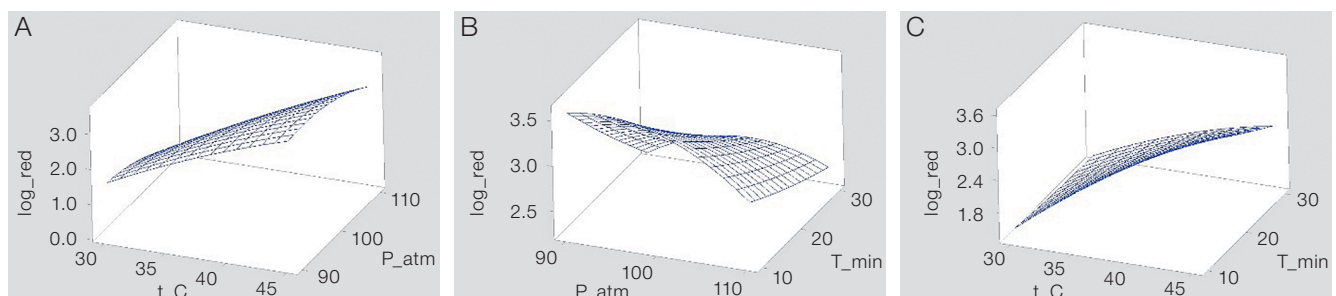


Figure prepared by the authors based on original data

Fig. 8. Response surface of the log-reduction parameter according to the regression model for the CDPAA group: A — P_{atm} and t_C at fixed T (10 min); B — P_{atm} and T_{min} at fixed t (45°C); C — t_C and T_{min} at fixed P (94 atm); t_C — temperature, °C; P_{atm} — pressure, atm; T_{min} — exposure duration, min; log_red — response: log_{10} -reduction (log_{10} units)

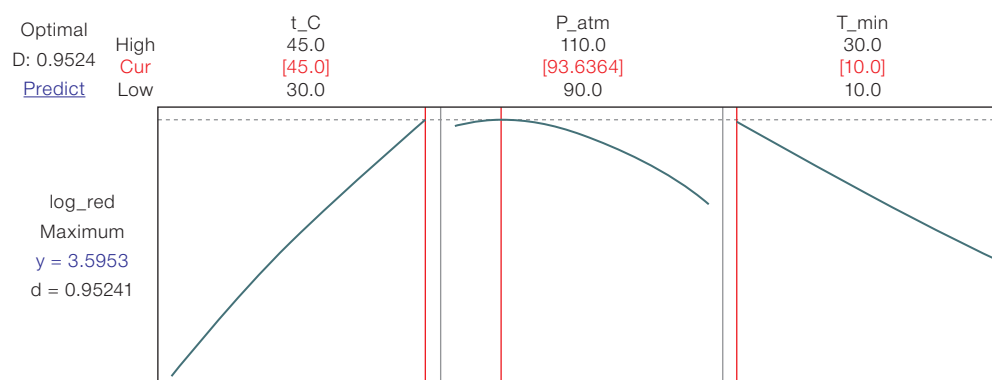


Figure prepared by the authors based on original data

Fig. 9. Optimization plot of treatment conditions for the CDPAA group: t_°C — temperature, °C; P_atm — pressure, atm; T_min — exposure duration, min; log_red — log₁₀-reduction; D — composite desirability; vertical lines — optimal factor levels

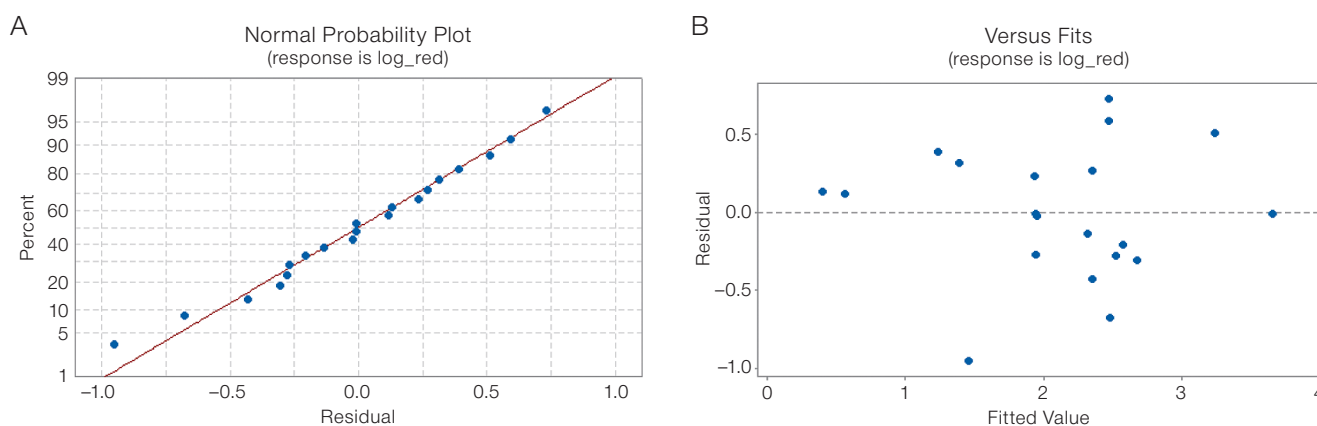


Figure prepared by the authors based on original data

Fig. 10. Residuals diagnostics for the log-reduction model (CDPAA group): A — normal probability plot (X-axis: residuals, log₁₀ units; Y-axis: percentage, %); B — residuals vs. predicted values plot (X-axis: predicted log₁₀-reduction values, log₁₀ units; Y-axis: residuals, log₁₀ units)

values (Fig. 10B) revealed no deviations from normal distribution or signs of heteroscedasticity.

Validation of optimal sterilization regimes

In order to confirm the effectiveness of the established optimal sterilization conditions and evaluate the method reproducibility, validation experiments with ten independent replicates for each regime were conducted. In the CDHP regime (37.9°C, 120 atm, 30 min, 200 ppm H₂O₂), the mean log-reduction was 4.4 ± 0.3 with a coefficient of variation of 7.3%. For the CDPAA regime (45°C, 94 atm, 10 min, 50 ppm PAA), the mean log-reduction was 6.0 ± 0.3 , with a coefficient of variation of 4.9%. In all experiments, the log-reduction values exceeded the detection limit of the method (1 CFU/mL).

The data distribution in both groups conformed to the normal distribution (AD: $p > 0.05$), allowing the use of a t -test to compare the effectiveness of the regimes. It was found that the CDPAA regime enables a statistically significant higher spore inactivation compared to CDHP (mean difference 1.60 (log-reduction); 95% CI: [1.31; 1.89]; $p < 0.001$).

DISCUSSION

Our comparative evaluation of the sterilizing effect of supercritical CO₂ and its combinations with oxidizing agents on *B. subtilis* spores found that the use of CO₂ alone (CD regime) did not lead to a significant reduction in the spore viability: in all tested parameter combinations, the inactivation level did not exceed 0.8 log orders. The introduction of oxidizing agents — hydrogen peroxide (CDHP) and peracetic acid (CDPAA) — yielded fundamentally different results. According to validation series data, for the CDHP regime, the mean reduction was 4.42 ± 0.32 log orders (CV = 7.30%) at 37.9°C, 120 atm, 30 min, compared to 6.02 ± 0.30 log orders (CV = 4.98%) at 45°C, 94 atm, 10 min, for CDPAA.

The coefficients of variation were calculated from a series of 10 independent replicates for regimes with oxidizing agents; for the CD regime, separate validation experiments were not conducted since no optimal conditions providing a high degree of inactivation could be identified.

The four-fold or greater reduction of viable *B. subtilis* spores achieved in our study through treatment with scCO₂ and hydrogen peroxide indicates the potential of this approach for high-level disinfection of semi-critical medical devices [1, 13]. Such an inactivation level is considered acceptable in current international guidelines for ensuring the microbiological safety of devices where absolute sterility is not required but effective protection against most pathogens is necessary [13–15]. It should be emphasized that we primarily assessed spore inactivation; therefore, further analysis of antimicrobial activity against other clinically relevant microorganisms is required for practical implementation of the method.

The low effectiveness of *Bacillus subtilis* spore sterilization using pure CO₂, observed across the studied range of temperatures (35–60°C), pressures (70–120 atm), and exposure duration (1–2 h), is fully consistent with the results of several independent studies. In the work by Spilimbergo et al. [5] at 36°C, 80 atm, and extended exposure (up to 48 h), the log-reduction for *B. subtilis* spores was 0.5–0.9, which agrees with our values of 0.4–0.8 at significantly shorter treatment modes. Dillow et al. [16] demonstrated that at 205 bar (~200 atm), 34°C, and a 2-h exposure, the log-reduction for *Bacillus cereus* spores did not exceed 1–2 units. Only at 60°C with an increased duration of 4 h, a log-reduction of 5–8 units was achieved, which falls outside the scope of most regimes applicable to medical devices. White et al. [17] confirmed that even at 96–103 atm and 35–60°C following a 72-h exposure, the log-reduction for *B. subtilis* and *B. stearothermophilus* spores remained close to zero; significant inactivation was observed only upon the addition of oxidizers or water.

Altogether, these data confirms that spore sterilization using pure CO₂ is ineffective under mild and moderate regimes. In practice, such inactivation requires either an extreme increase in temperature or a significant extension of treatment exposure, which is often impractical or economically unfeasible when working with medical materials. In our study, the parameter range was selected based on the constraints of the experimental facility and the objectives related to the applicability of the method for processing medical devices, which aligns with real-world production conditions and the prospects for subsequent technology implementation.

The agreement between our obtained log-reduction values and the ranges reported in several independent studies for *Bacillus spp.* spores under similar conditions [5, 16, 17] may indicate a certain reproducibility of the low effectiveness of pure CO₂ within the studied regimes. Despite differences in experimental models, subjects, and protocol details, no significant discrepancies in the achieved level of inactivation were observed. However, definite conclusions regarding reproducibility require specifically designed interlaboratory studies with control of critical parameters.

It should be noted that we were unable to identify other publications on the use of the *B. subtilis* strain and comparable treatment conditions (200 ppm H₂O₂, temperature, pressure), except for the work by Warambourg

et al. [6], whose parameters were used as a reference when planning our experiments. In our study, the optimal process parameters (120 bar, 37.9°C, 30 min, 200 ppm H₂O₂) differed from those described by Warambourg et al. [6] (110 bar, 40°C, 20 min, 200 ppm H₂O₂), although being close in values. The overlap in the ranges of optimal conditions, determined independently, may be considered an indication of partial reproducibility of the “optimal sterilization window” for scCO₂ with H₂O₂ for *B. subtilis*. However, the achieved log-reduction values differed significantly (4.42 vs. 8.73), indicating limitations in quantitative reproducibility between different laboratories and underscoring the importance of standardizing experimental methods and reference materials.

Regarding the application of CDPA, the most similar conditions to the present study are found in the experiments by Setlow et al., where for *B. subtilis* at 35–55 ppm PAA, 35°C, 100 bar, and an exposure duration of 25 min, a reduction in viability of more than 6 log orders was observed [12]. In our study, the optimal parameters were 45°C, 94 bar, 10 min, 50 ppm PAA. Thus, while the ranges of concentration, pressure, and temperature that result in significant inactivation were similar, differences in exposure duration and protocol details preclude claims of reproducibility in the strict sense and complicate a direct quantitative comparison of effectiveness.

Despite the partial overlap of key parameters with those used in publications, a correct quantitative comparison of inactivation effectiveness remains challenging. The main reasons stem from differences in the specific strains used, the geometry and material of the substrate, the method and volume of inoculum application, the recovery procedure, as well as details of the experimental protocols. This variability underscores the need for further standardization and interlaboratory alignment of key parameters in scCO₂ sterilization research.

The available literature data on spore inactivation using scCO₂ in combination with oxidizing agents (hydrogen peroxide, peracetic acid) are highly heterogeneous. Most publications investigate different *Bacillus* strains (most commonly *B. atropheus*, *B. pumilus*, *G. stearothermophilus*), employ substantially different oxidizer concentrations, and utilize varied experimental protocols [18]. We were unable to identify any studies that simultaneously used low oxidizer concentrations, conditions comparable to the present study in terms of temperature and pressure, and *B. subtilis* as the test organism. This fragmentation and lack of standardization hinder a correct quantitative comparison of inactivation effectiveness between the results of the present work and most published data. The necessity for further research using identical biological materials and harmonized regimes is emphasized in a number of recent reviews [12, 18].

Our analysis of the contribution of individual factors revealed that in the CDHP regime, pressure exerted a highly significant influence both in linear ($p < 0.05$) and quadratic ($p < 0.001$) terms, with the quadratic effect being the most pronounced. In the CDPA regime, the linear effect of pressure did not reach statistical significance ($p > 0.226$),

which is likely associated not only with differences in the chemical properties of the oxidizing agents but also with characteristics of the matrix and the local availability of the sterilizing agent [9]. Our data are partially consistent with those of Hossain et al., where pressure was the determining factor for bacterial inactivation under pure scCO_2 treatment conditions; however, that study primarily investigated vegetative forms rather than spores [8]. According to Zhang et al., pressure enhances the solubility and penetration of CO_2 through cellular barriers, although the efficiency of this process can vary significantly for spores [7, 9].

Temperature demonstrated consistent significance in both models: for CDHP — as both a linear ($p < 0.01$) and quadratic ($p < 0.05$) effect; for CDPA — as the dominant linear factor ($p < 0.05$). This highlights the leading role of temperature when oxidizers are added to overcome spore barriers. The significance of the temperature factor identified in our study for the regime with peracetic acid aligns with the data by Zhang et al., according to whom an increase in temperature enhances CO_2 diffusion and the fluidity of cellular structures [7].

Exposure duration was statistically significant only for CDHP ($p < 0.05$) but not for CDPA ($p > 0.407$), which is also consistent with literature data indicating a relatively smaller contribution of exposure duration compared to pressure and temperature [7, 9]. The faster action of peracetic acid may explain the reduced dependence on exposure duration.

The pressure–temperature interaction was statistically significant for both regimes (CDHP: $p < 0.05$; CDPA: $p < 0.05$), indicating a synergistic effect between these parameters. It appears that at pressures above the critical point for CO_2 , diffusion is primarily governed by pressure, while increasing temperature further enhances membrane fluidity and activates oxidative processes [7, 8].

In our study, rapid decompression after treatment was intentionally excluded to minimize the contribution of mechanical damage to spores and to obtain a more objective assessment of the chemical action of scCO_2 and oxidizing agents. This approach is justified by the results of several studies demonstrating that rapid decompression can have a significant effect on inactivation through physical effects unrelated to the action of the agent itself [5, 7, 8]. In our opinion, this approach provides more reliable data on inactivation mechanisms.

The validation experiments showed log-reduction values substantially exceeding the upper bounds of the prediction intervals calculated from the main series during the optimization of the regimes. This discrepancy is attributed not only to the application of the optimized regimes but also to the use of different microbiological approaches in the two series. Thus, during model construction, the initial spore concentration was lower (to ensure stable operation of the automatic counter), whereas validation was performed at the normative load ($N_0 \geq 10^6$) required for SAL calculation [14, 15].

Our study is not devoid of several limitations that should be considered when interpreting and practically applying the data obtained. The work was conducted on a single laboratory facility with a maximum pressure of 120 atm, which limits the range of investigated regimes. The experiments were performed with only one model organism (*Bacillus subtilis* subsp. *spizizenii* ATCC 6633) and on titanium discs. Therefore, the effectiveness of the method when applied to other microorganisms and materials was not evaluated. The result reproducibility was determined only within a single laboratory setting, without interlaboratory comparisons. In addition, we did not investigate the effect of sterilization on the functional properties and biocompatibility of medical devices. All these limitations should be considered when planning further work and translating laboratory results into practice.

CONCLUSIONS

During the conducted experimental studies using supercritical carbon dioxide (scCO_2), including pure scCO_2 , scCO_2 with hydrogen peroxide (H_2O_2), and scCO_2 with peracetic acid (PAA), regimes for the inactivation of *Bacillus subtilis* spores on titanium discs were validated. Optimal conditions for the low-temperature processing of thermally sensitive medical materials, compliant with modern international requirements, were experimentally substantiated. The scCO_2 with peracetic acid regime achieves a sterility assurance level (SAL) of $\leq 10^{-6}$, which is required for the sterilization of medical devices. The scCO_2 with hydrogen peroxide regime exceeds $\text{SAL} \leq 10^{-4}$, corresponding to high-level disinfection. The use of pure scCO_2 alone proved insufficiently effective (maximum 0.8 log-reduction).

References

1. Rowan NJ, Kremer T, McDonnell G. A review of Spaulding's classification system for effective cleaning, disinfection and sterilization of reusable medical devices: viewed through a modern-day lens that will inform and enable future sustainability. *Science of the Total Environment*. 2023;878:162976. <https://doi.org/10.1016/j.scitotenv.2023.162976>
2. Spaulding AB, Watson D, Dreyfus J, Heaton P, Grapentine S, Bendel-Stenzel E, et al. Epidemiology of bloodstream infections in hospitalized children in the United States, 2009–2016. *Clinical Infect Diseases*. 2019;69(6):995–1002. <https://doi.org/10.1093/cid/ciy1030>
3. Stewart S, Robertson C, Pan J, Kennedy S, Dancer S, Haahr L, et al. Epidemiology of healthcare-associated infection reported from a hospital-wide incidence study: considerations for infection prevention and control planning. *Journal of Hospital Infection Society*. 2021;114:10–22. <https://doi.org/10.1016/j.jhin.2021.03.031>
4. Arutyunov SD, Yanushevich OO, Korsunsky AM, Podporin MS, Salimon AI, Romanenko II, et al. Comparative analysis of the effectiveness of modern methods of sterilization of instruments and the place of gas-dynamic treatment with carbon dioxide. *Russian Journal of Stomatology*. 2022;15(1):12–9 (In Russ.). <https://doi.org/10.17116/rosstomat2022150112>
5. Spilimbergo S, Bertucco A, Lauro FM, Bertoloni G. Inactivation of *Bacillus subtilis* spores by supercritical CO_2 treatment. *Innovative Food Science and Emerging*

- Technologies*. 2003;4(2):161–5.
[https://doi.org/10.1016/S1466-8564\(02\)00089-9](https://doi.org/10.1016/S1466-8564(02)00089-9)
6. Warambourg V, Mouahid A, Crampon C, Galinier A, Claeys-Bruno M, Badens E. Supercritical CO₂ sterilization under low temperature and pressure conditions. *The Journal of Supercritical Fluids*. 2023;203:106084.
<https://doi.org/10.1016/j.supflu.2023.106084>
 7. Zhang J, Davis TA, Matthews MA, Drews MJ, LaBerge M, An YH. Sterilization using high-pressure carbon dioxide. *The Journal of Supercritical Fluids*. 2006;38(3):354–72.
<https://doi.org/10.1016/j.supflu.2005.05.005>
 8. Hossain MS, Nik Ab Rahman NN, Balakrishnan V, Alkarkhi AFM, Rajion ZA, Ab Kadir MO. Optimizing supercritical carbon dioxide in the inactivation of bacteria in clinical solid waste by using response surface methodology. *Waste Management*. 2015;43:402–9.
<https://doi.org/10.1016/j.wasman.2015.01.003>
 9. Qiu QQ, Leamy P, Brittingham J, Pomerleau J, Kabaria N, Connor J. Inactivation of bacterial spores and viruses in biological material using supercritical carbon dioxide with sterilant. *Journal of Biomedical Materials Research B Applied Biomaterials*. 2009;91B(2):572–8.
<https://doi.org/10.1002/jbm.b.31431>
 10. Salimon AI, Statnik ES, Kan Y, Yanushevich OO, Tsarev VN, Podporin MS, et al. Comparative study of biomaterial surface modification due to subcritical CO₂ and autoclave disinfection treatments. *The Journal of Supercritical Fluids*. 2022;191:105789.
<https://doi.org/10.1016/j.supflu.2022.105789>
 11. McFarland J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *JAMA*. 1907;49(14):1176–8.
<https://doi.org/10.1001/jama.1907.25320140022001f>
 12. Setlow B, Korza G, Blatt KMS, Fey JP, Setlow P. Mechanism of *Bacillus subtilis* spore inactivation by and resistance to supercritical CO₂ plus peracetic acid. *Journal of Applied Microbiology*. 2016;120(1):57–69.
<https://doi.org/10.1111/jam.12995>
 13. von Woedtke T, Kramer A. The limits of sterility assurance. *GMS Krankenhhyg Interdiszip*. 2008;3(3):Doc19.
 14. Rutala WA, Weber DJ. Sterilization, high-level disinfection, and environmental cleaning. *Infectious Disease Clinics of North America*. 2011;25(1):45–76.
<https://doi.org/10.1016/j.idc.2010.11.009>
 15. Shenoy ES, Weber DJ, McMullen K, Rubin Z, Sampathkumar P, Schaffzin JK, et al. Multisociety guidance for sterilization and high-level disinfection. *Infection Control Hospital Epidemiology*. 2025.
<https://doi.org/10.1017/ice.2025.41>
 16. Dillow AK, Dehghani F, Hrkach JS, Foster NR, Langer R. Bacterial inactivation by using near- and supercritical carbon dioxide. *PNAS*. 1999;96(18):10344–8.
<https://doi.org/10.1073/pnas.96.18.10344>
 17. White A, Burns D, Christensen TW. Effective terminal sterilization using supercritical carbon dioxide. *Journal of Biotechnology*. 2006;123(4):504–15.
<https://doi.org/10.1016/j.jbiotec.2005.12.033>
 18. Soares GC, Learmonth DA, Vallejo MC, Davila SP, González P, Sousa RA, et al. Supercritical CO₂ technology: The next standard sterilization technique? *Material Science and Engineering: C*. 2019;99:520–40.
<https://doi.org/10.1016/j.msec.2019.01.121>

Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. The primary contributions are distributed as follows: Ramaz Sh. Gvetadze — administrative management of the project, manuscript review and editing; Mariam S. Galstyan — conducting experiments on the facility, processing primary data, writing the initial version of the article, and preparing illustrative material; Yaser N. Kharakh — developing the statistical design of the study, statistical analysis, and results interpretation; Petr Yu. Kolesnikov — conducting microbiological research, cultivating microorganisms, and performing microbiological analysis; Levon G. Kirakosyan — technical support for experiments, conducting validation experiments, and writing the methodology and results sections; Mikhail S. Podporin — planning experiments to optimize modes, and scientific and methodological support; Viktor N. Tsarev — concept of the microbiological part of the study, provision of laboratory resources, and scientific editing; Sergey D. Arutyunov — study conceptualization, methodology development, and approval of the final version of the manuscript for publication.

AUTHORS

Ramaz Sh. Gvetadze, Dr. Sci. (Med.), Professor, Academician of RAS
<https://orcid.org/0000-0003-0508-7072>
ramaz-gvetadze@yandex.ru

Mariam S. Galstyan
<https://orcid.org/0000-0002-3372-5775>
galstyan.mariam17@gmail.com

Yaser N. Kharakh, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-7181-8211>
c.kharakh@gmail.com

Petr Yu. Kolesnikov
<https://orcid.org/0000-0003-2947-726X>
petrs8@mail.ru

Levon G. Kirakosyan, Cand. Sci. (Med.)
<https://orcid.org/0000-0001-5380-1475>
dr.lkirakosyan@gmail.com

Mikhail S. Podporin, Cand. Sci. (Med.)
<https://orcid.org/0000-0003-1737-0887>
podporin.mikhail@yandex.ru

Viktor N. Tsarev, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0002-3311-0367>
nikola777@rambler.ru

Sergey D. Arutyunov, Dr. Sci. (Med.), Professor
<https://orcid.org/0000-0001-6512-8724>
sd.arutyunov@mail.ru

<https://doi.org/10.47183/mes.2025-347>

HONEY-BASED HYDROGEL: A PROMISING NEW-GENERATION ANTIMICROBIAL COATING?

Ekaterina N. Karpina¹, Elena V. Fedotova^{1,2}, Faliya F. Zaripova¹, Denis V. Krivorotov², Andrey S. Radilov²

¹ITMO University, St. Petersburg, Russia

²Research Institute of Hygiene, Occupational Pathology and Human Ecology, Kuzmolovsky, Leningrad region, Russia

Introduction. The development of hydrogel coatings is a promising research direction in burn injury therapy. Honey-based hydrogels possess unique physicochemical characteristics due to being transparent, capable of effectively absorbing exudate, and exhibiting low pH values. All these properties make them ideal candidates for application not only in cosmetology but also in medicine for the transdermal delivery of various medicinal substances.

Objective. Synthesis of honey-based hydrogels and comparative analysis of their physicochemical characteristics and antibacterial activity.

Materials and methods. Hydrogels were synthesized based on chitosan and carbopol with addition of 25, 50, and 75 wt % of white honey. The following parameters were investigated: viscosity (using a Brookfield rotational viscometer); spreadability (by a compression method); degree of sample swelling; stability (by centrifugation); honey release from the sample (using an ERWEKA DT-820 tester); analysis of the quantitative honey content in the buffer (by spectrophotometry). The study of *in vitro* antibacterial properties was conducted using *Candida albicans* ATCC 64550 (a yeast-like fungus), *Staphylococcus aureus* ATCC 25913 (a gram-positive bacterium), *Escherichia coli* ATCC 25922 (a gram-negative bacterium), *Acinetobacter baumannii* 897 (a gram-negative bacterium), *Enterobacter cloacae* ATCC 13047 (a gram-negative bacterium), *Klebsiella pneumoniae* ATCC 19606 (a gram-negative bacterium). Statistical data processing was performed using the DeltaX 3.0 software.

Results. In terms of viscosity, the hydrogel based on 1% Carbopol 940 with a honey concentration of 25 wt % outperformed other gels. The samples based on 1% Carbopol ETD 2020 with a honey concentration of 50 wt % exhibited the lowest spreadability. The highest sorption capacity 1 h and 3 h after the onset of testing was demonstrated by the sample based on 3.5% low-viscosity chitosan with a honey concentration of 25 wt %. The highest honey release was shown by the sample based on 10% low-viscosity chitosan with a honey concentration of 50 wt %.

Conclusions. The conducted study of physicochemical and antibacterial properties render chitosan-based hydrogels promising for further investigation. Hydrogel samples with a 75% honey concentration failed the stability test, which make them unsuitable for use as a therapeutic delivery system. Based on the results obtained, a mixed hydrogel based on Carbopol 940 and 3.5% low-viscosity chitosan is recommended for further research.

Keywords: regenerative medicine; burns; honey; hydrogel; wound dressing

For citation: Karpina E.N., Fedotova E.V., Zaripova F.F., Krivorotov D.V., Radilov A.S. Honey-based hydrogel: A promising new-generation antimicrobial coating? *Extreme Medicine*. 2026;28(1):146–156. <https://doi.org/10.47183/mes.2025-347>

Funding: the study was carried out without sponsorship.

Compliance with the ethical principles: the study did not require approval by an ethics committee.

Potential conflict of interest: Andrey S. Radilov is a member of the editorial board of *Extreme Medicine*. The other co-authors declare no conflict of interest.

✉ Elena V. Fedotova arabka2008@mail.ru

Received: 09 Jul 2025 **Revised:** 25 Sep. 2025 **Accepted:** 29 Oct. 2025 **Online first:** 27 Dec. 2025

УДК 615.45

МЕДОВЫЙ ГИДРОГЕЛЬ — ПЕРСПЕКТИВНОЕ АНТИБАКТЕРИАЛЬНОЕ ПОКРЫТИЕ НОВОГО ПОКОЛЕНИЯ?

Е.Н. Карпина¹, Е.В. Федотова^{1,2}, Ф.Ф. Зарипова¹, Д.В. Криворотов², А.С. Радилов²

¹Национальный исследовательский университет ИТМО, Санкт-Петербург, Россия

²Научно-исследовательский институт гигиены, профпатологии и экологии человека Федерального медико-биологического агентства, Ленинградская область, Россия

Введение. Разработка гидрогелевых покрытий — это перспективное направление для исследований в области терапии ожоговых повреждений. Гидрогели на основе меда обладают уникальными физико-химическими характеристиками: они прозрачны, хорошо впитывают экссудат и имеют низкие значения pH. Все это делает их идеальными кандидатами для применения не только в косметологии, но и в медицине для трансдермальной доставки различных лекарственных веществ.

Цель. Получение гидрогелей на основе полимеров и меда, сравнительный анализ их физико-химических характеристик и определение антибактериальной активности.

Материалы и методы. Использовали полученные гидрогели из хитозана и карбопола, содержащие в своем составе липовый мед в весовых концентрациях 25, 50 и 75%. Исследовали показатели: вязкость (на ротационном вискозиметре Brookfield); растекаемости (методом прессования); степень набухания образцов; стабильность (методом центрифугирования); высвобождение меда из образцов (в тесте высвобождения с помощью ERWEKA DT-820); анализ количественного содержания меда в буфере (методом спектрофотометрии). Исследование антибактериальных свойств *in vitro* проводили с использованием: *Candida albicans* ATCC 64550 — дрожжеподобный грибок; *Staphylococcus aureus* ATCC 25913 — грамположительная бактерия; *Escherichia coli*

© E.N. Karpina, E.V. Fedotova, F.F. Zaripova, D.V. Krivorotov, A.S. Radilov, 2025

ATCC 25922 — грамотрицательная бактерия; *Acinetobacter baumannii* 897 — грамотрицательная бактерия; *Enterobacter cloacae* ATCC 13047 — грамотрицательная бактерия; *Klebsiella pneumoniae* ATCC 19606 — грамотрицательная бактерия. Статистическая обработка данных выполнена с помощью программного обеспечения DeltaX 3.0.

Результаты. Наилучший образец по показателю вязкости — гидрогель на основе 1% карбопола 940 с концентрацией меда 25%. Наименьшей растекаемостью обладали образцы на основе 1% карбопола ETD 2020 с концентрацией меда 50%. Наилучшая сорбционная емкость через 1 ч после начала испытания и через 3 ч продемонстрирована у образца на основе 3,5% низковязкого хитозана с концентрацией меда 25%. Наилучший результат высвобождения меда показал образец на основе 10% низковязкого хитозана с концентрацией меда 50%.

Выводы. Наиболее перспективными для дальнейшего изучения образцами по результатам исследования физико-химических и антибактериальных свойств признаны гидрогели на основе хитозана. В результате сравнительного анализа было выявлено, что образцы гидрогелей с концентрацией меда 75% не проходят испытание на стабильность, что делает невозможным их применение в качестве терапевтической системы доставки. На основании исследования физико-химических показателей исследованных гидрогелей, а также их антибактериальных свойств можно рекомендовать провести испытания смешанного гидрогеля на основе карбопола 940 и 3,5% низковязкого хитозана.

Ключевые слова: регенеративная медицина; ожоги; мед; гидрогель; раневое покрытие

Для цитирования: Карпина Е.Н., Федотова Е.В., Зарипова Ф.Ф., Криворотов Д.В., Радилов А.С. Медовый гидрогель — перспективное антибактериальное покрытие нового поколения? *Медицина экстремальных ситуаций*. 2026;28(1):146–156. <https://doi.org/10.47183/mes.2025-347>

Финансирование: исследование выполнено без спонсорской поддержки.

Соответствие принципам этики: исследование не требует одобрения этического комитета.

Потенциальный конфликт интересов: А.С. Радилов — член редакционной коллегии журнала «Медицина экстремальных ситуаций». Остальные авторы заявляют об отсутствии конфликта интересов.

✉ Федотова Елена Викторовна arabka2008@mail.ru

Статья поступила: 09.07.2025 **После доработки:** 25.09.2025 **Принята к публикации:** 29.10.2025 **Online first:** 27.12.2025

INTRODUCTION

Burns are one of the most common types of injuries, both in domestic and industrial settings. In combustiolog, burns are classified into chemical, thermal, radiation, and combined types. Susceptibility to infection by various bacteria and the need to stimulate skin regeneration are unifying factors for all these groups.

Hydrogels possess a number of properties that underpin their advantages for use in combustiolog. These include absorption capacity, biodegradability, affinity to the extracellular matrix ensuring biocompatibility, non-adhesiveness, and the ability to deliver antimicrobial components directly to the site of injury [1–3]. These properties determine the potential efficacy of hydrogel applications in burn care.

Hydrogels based on smart polymers capable of responding to external stimuli, such as pH level, temperature, and reactive oxygen species (ROS) concentration, are increasingly attracting the research attention [3]. One promising direction involves the development of synthetic hydrogels with programmed release of bioactive substances capable of influencing the pathogenesis of the wound healing process [4].

The ability of hydrogels to absorb fluids and swell allows a specific moisture level to be maintained. This facilitates control over exudate levels and ensures necessary solubilization. The biocompatibility of hydrogel-based polymers is important for mitigating the risk of immune response induction and rejection by the body.

Polymeric hydrogel coatings effectively bind with various bioactive and pharmaceutical substances, enabling

the delivery of antimicrobial, anti-inflammatory, analgesic, and other therapeutic agents directly to the affected area [5]. Certain hydrogels can also exhibit intrinsic antibacterial properties, such as synthetic polymeric hydrogels based on poly(2-hydroxyethyl methacrylate) [4]. Maikovych et al. also demonstrated the antibacterial effect of hydrogels based on gelatin cross-linked with polyethylene glycol against *S. aureus* and *E. coli* [6].

As a rule, hydrogels for burn care are fabricated from natural polymers such as chitosan, sodium alginate, collagen, hyaluronic acid, cellulose, gelatin, etc. Synthetic polymers including polyvinyl alcohol, polyacrylamide, polyethylene glycol, and carbopol may also hold promise in this field. Currently, hybrid hydrogels are among the most widely used. These are created by combining two or more synthetic and natural polymers. By leveraging the physicochemical properties of the constituent polymers, the application scope of such hybrid hydrogels can be tailored [7]. One example of such combinations is a hydrogel based on carboxymethyl cellulose and chitosan, formed via a Schiff base reaction. This hydrogel was recommended for the healing of deep burns due to its excellent tissue adhesion [8].

The antibacterial properties of honey have long been of interest to researchers. Its effectiveness against both pathogenic and non-pathogenic microorganisms, including bacteria, yeasts, and fungi, has been demonstrated in numerous scientific studies. The antimicrobial activity honey is associated with low pH values (acidic environment), the content of hydrogen peroxide, sugars, and protein [9]. The high content of sugar creates a protective barrier to inhibit the development

of microorganisms. The reduction in the amount of unbound water molecules in honey leads to suppressed microbial growth and proliferation [10]. The antifungal properties of honey are also linked to its physicochemical characteristics. Phenolic compounds present in honey are believed to contribute to protein denaturation, significantly destabilizing cellular membranes and ultimately leading to their disruption in fungi [11, 12].

The anti-inflammatory properties of honey are equally relevant to burn medicine. Inflammation is a process driven by immune response to various injuries or the invasion of foreign pathogenic microorganisms. The mitigating effect of honey on the inflammatory process is thought to be associated with its phenolic constituents. Galangin (one of the honey flavonoids) inhibits the activity of cyclooxygenase (COX) and lipoxygenase-2, reduces the expression of cyclooxygenase-2 (COX-2), and restrains the action of polygalacturonase [13]. Some authors attribute the effect of honey on the molecular mechanisms underlying the immune response to the induction of NF- κ B (a transcription factor), TNF- α (tumor necrosis factor alpha), and the pro-inflammatory cytokines IL-6 (interleukin-6) and IL-1 β (interleukin-1 β) [14].

In addition to its antimicrobial properties, the wound-healing capacity of honey is also noted. Its activity can be both bacteriostatic and bactericidal, depending on the type of honey. When honey comes into contact with a wound, it reduces protease activity in the damaged area, increases oxygen release from hemoglobin, stimulates fibroblasts and macrophages. The production of hydrogen peroxide stimulates vascular endothelial growth factor and sterilizes the wound. Thus, honey can be used as a component incorporated into wound dressings [15].

In order to prevent the development of pathogenic microorganisms during burn healing, it is essential to maintain an optimal level of exudate. Honey possesses significant osmotic potential, which promotes the

release of lymphatic fluid. This fluid, in turn, cleanses and disinfects the wound and aids in the removal of foreign particles from the wound bed. Due to its osmotic potential and the ability to absorb moisture, honey as a wound dressing significantly reduces the risk of swelling and skin wrinkling, protecting the wound from over-hydration [16, 17].

In this study, we set out to develop hydrogels based on polymers and honey, conduct a comparative analysis of their physicochemical characteristics, and determine their antibacterial activity.

MATERIALS AND METHODS

The starting compounds were Far Eastern white (linden) honey purchased from the store "Honey & Confiture" (2023 harvest, article number: 00000001221 with a quality certificate from the supplier), high-viscosity chitosan (3.5%, Sigma-Aldrich), low-viscosity chitosan (3.5%, Sigma-Aldrich), Carbopol 940 (1%, Lubrizol), and Carbopol ETD 2020 (1%, Acros Organics).

Preparation of chitosan- and carbopol-based hydrogels

Hydrogels were prepared according to a standard protocol as follows. A weighed sample of chitosan was dissolved in a weak acetic acid solution using a Heidolph Silent Crusher M homogenizer (Germany) at a speed of 9000 rpm (Fig. 1). A weighed sample of Carbopol was dissolved in water using a homogenizer at 9000 rpm. The resulting polymer solutions were adjusted to a neutral pH (pH = 6.0–7.0) with triethanolamine (TEA) under continuous stirring. pH was monitored using a pH meter (pH-150MI, Russia). Polyethylene glycol (PEG) was used as a cross-linking agent and preservative. The resulting polymer solutions were left to stabilize and swell for 24 h in a refrigerator.

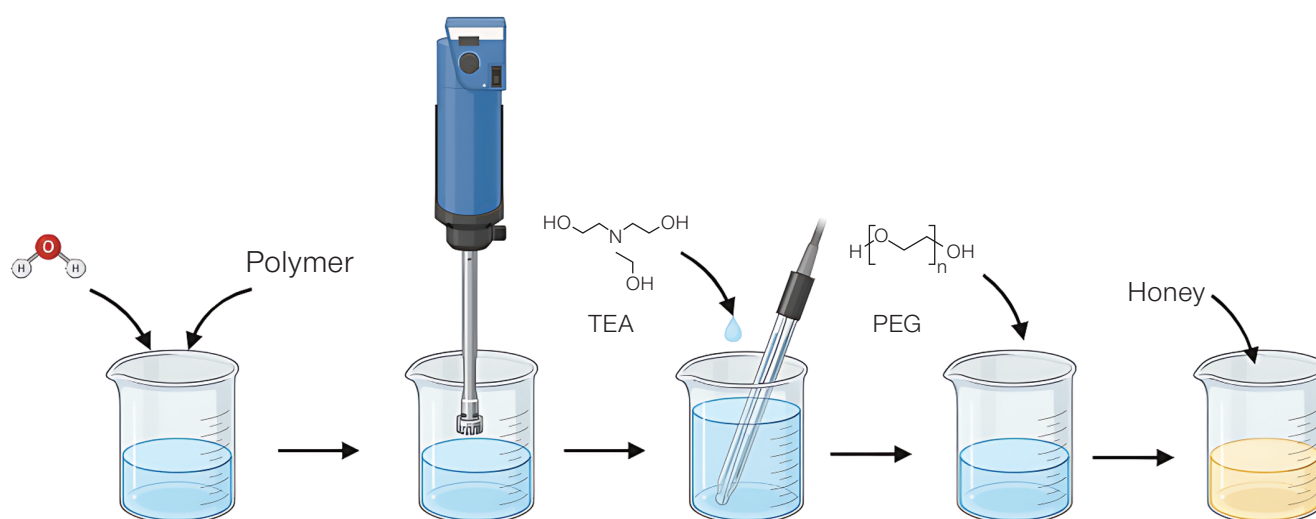


Figure prepared by the authors using their own data

Fig. 1. Protocol for the preparation of honey-containing hydrogel samples

Preparation of honey-containing hydrogels

White (linden) honey was liquefied in a drying oven at a temperature of 45°C and added to the hydrogels under constant stirring (Fig. 1). The following honey concentrations were selected for each polymer solution: 25, 50, and 75 wt %. The resulting hydrogels were stored in a refrigerator for 24 h to stabilize and ensure uniform honey distribution.

Viscometric analysis

The viscosity of the hydrogels was investigated using a Brookfield LV-SSR Brinell Viscometer rotational viscometer (China). The experiment for each sample was repeated three times. The average viscosity value was calculated.

Sorption capacity investigation

A phosphate buffer with a pH of 5.5, corresponding to the optimal pH of human skin, was prepared as follows: a 13.61 g sample of potassium dihydrogen phosphate was dissolved in water in a 1000 mL volumetric flask. A 3.58 g sample of disodium hydrogen phosphate was dissolved in water, bringing the solution to the 100 mL mark. Then, 964 mL of the potassium dihydrogen phosphate solution was mixed with 36 mL of the disodium hydrogen phosphate solution.

Dry samples were immersed in the phosphate buffer, ensuring their complete submergence. The samples were left for 1 h followed by draining excess liquid and weighing. The samples were then immersed in buffer again and weighed after another 2 h. The experiment was repeated three times for each sample; the average value for each sample was calculated.

The degree of swelling was determined by the amount of fluid absorbed per 1 g of dry substance, according to formula (1):

$$Q = \frac{m - m_0(1 - \gamma)}{m_0(1 - \gamma)}, \quad (1)$$

where Q — hydrogel sorption capacity, g/g; m — mass of the swollen sample, g; m_0 — initial mass of the sample, g; γ — moisture content of the gel sample, mass fraction.

The moisture content of the gel sample, mass fraction, equals:

$$W\% = \frac{m_0 - m_c}{m_0} \times 100\%, \quad (2)$$

where m_0 — initial mass of the sample, g; m_c — mass of the sample dried to constant weight, g.

Spreadability study

The spreadability of the hydrogels was assessed using a compression method (Fig. 2) [18]. A 0.5 g sample of each formulation was placed between two glass slides. A 5 g weight was placed on the top slide, and, following 5 min, the diameters of the formed discs were measured using a ruler. Measurements were taken along three different axes. The experiment was repeated three times for each sample, with the average diameter being calculated for each formulation.

Hydrogel stability

Hydrogel stability was evaluated using a centrifugation method (Fig. 3). Each 15 mL sample was centrifuged (ZJMZYM Timing Medical Centrifuge, China) at 4000 rpm for 10 min. This was followed by a visual assessment of the samples for precipitate formation or phase separation. The experiment was repeated three times for each sample.

In accordance with the State Pharmacopoeia,¹ a phosphate buffer with pH = 5.5 was selected as the model medium. The study utilized a ERWEKA DT-820 dissolution tester (Germany). A 500 mL volume of the dissolution medium was poured into a vessel thermostated in a water bath to a temperature of $32.0 \pm 0.5^\circ\text{C}$. The sample holder was immersed into the vessel containing the dissolution medium, after which the apparatus with a rotational mechanism was started and operated for 4 h.

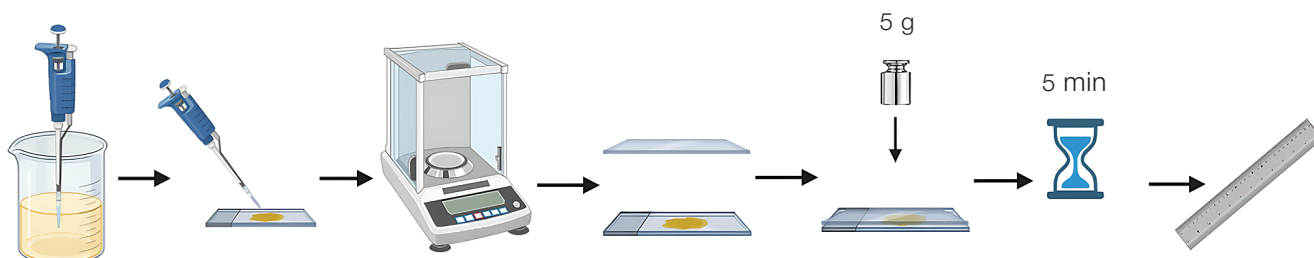


Figure prepared by the authors using their own data

Fig. 2. Methodology for spreadability testing

¹ General pharmacopoeial monograph "Buffer Solutions". OFS.1.3.0003 "State Pharmacopoeia of the Russian Federation. XV edition". Accessed from the reference-legal system "Consultant Plus" (access date 21.07.2025).

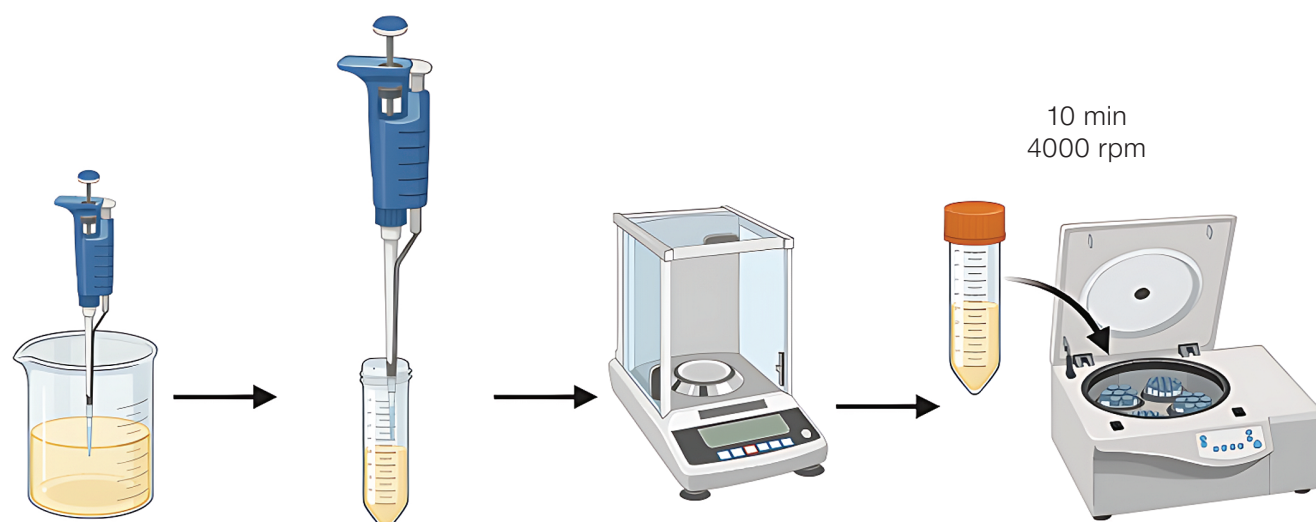


Figure prepared by the authors using their own data

Fig. 3. Methodology for stability testing. Honey release test from hydrogels

Samples were collected at hourly intervals. The samples were withdrawn from the mid-height region of the dissolution medium at a distance no closer than 10 mm from the inner wall of the vessel. The experiment was repeated three times for each sample, and the average value of the released substance was calculated for each formulation.

Spectrophotometric analysis of released honey

Quantitative analysis of honey in the buffer was performed using a Cary 100 UV Vis spectrophotometer (Agilent Technologies, USA). The absorption of the released honey was measured at a λ_{max} of 340 nm (flavonoid peak).

Based on the obtained absorption values, the concentration of the active substance in each collected sample was calculated using formula (3):

$$c = \frac{A_x \times C_{st}}{A_{st}}, \quad (3)$$

A_x , A_{st} — the absorbance values of the test solution and the standard solution, respectively; C_{st} — concentration of the active substance in the standard solution, g/mL.

In vitro antibacterial activity study

The following microorganisms were selected to study the antibacterial properties of the obtained samples: *Candida albicans* ATCC 64550 — a yeast-like fungus; *Staphylococcus aureus* ATCC 25913 — a gram-positive bacterium; *Escherichia coli* ATCC 25922 — a gram-negative bacterium; *Acinetobacter baumannii* 897 —

a gram-negative bacterium; *Enterobacter cloacae* ATCC 13047 — a gram-negative bacterium; *Klebsiella pneumoniae* ATCC 19606 — a gram-negative bacterium. Mueller-Hinton agar (Research Center for Pharmacotherapy) was used as the nutrient medium for cultivating microorganisms via the disk-diffusion method. Nutrient Medium No. 1 GRM (State Research Center for Applied Microbiology and Biotechnology) was used for preliminary control of microbial contamination of the emulsions. Experiments to evaluate antibacterial properties were performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI)² and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).³

The agar was inoculated using streaking motions in three directions with a microbial suspension prepared in sterile physiological saline (density 5×10^8 CFU/mL or 0.5 McFarland standard) to achieve a confluent lawn. Fifteen minutes after inoculation, disks impregnated with the hydrogel samples were placed in Petri dishes, which were then incubated with the microorganisms at 35 °C for 20 h. Upon completion of the incubation period, the diameters of the growth inhibition zones were measured with an accuracy of 1 mm. This was achieved by placing the Petri dishes upside down on a dark surface and illuminating them with a lamp at a 45-degree angle.⁴

Statistical data processing

Statistical data processing was performed using the DeltaX 3.0 software. A one-sample Student's *t*-test was used for result analysis. Data are presented as mean value and standard deviation (SD).

² CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed., CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.

³ EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing Version 12.0 (January 2024). www.eucast.org

⁴ Methodological Guidelines. MUK 4.2.1890-04 "Control Methods. Biological and Microbiological Factors. Determination of Microorganism Susceptibility to Antibacterial Drugs" (approved by the Chief State Sanitary Physician of the Russian Federation on 04.03.2004). Accessed via the "Consultant Plus" system. (Access date 25.05.2025).

RESULTS AND DISCUSSION

In order to compare the physicochemical properties of the hydrogels, samples with different honey weight concentrations (25, 50, and 75 wt %) were prepared. Visual assessment revealed that all samples with 25 and 50 wt % honey concentrations were similarly transparent and homogeneous, possessing a slight honey odor, and having a yellow (for 25 wt % concentration samples) or yellowish-brown color (for 50 wt % concentration samples). No mechanical inclusions were detected in these samples. Chitosan-based and carbopol-based samples with a 50 wt % honey concentration exhibited a viscous liquid consistency, unlike the carbopol-based samples with a 25 wt % honey concentration. The carbopol-based samples with a 25 wt % honey concentration had a gel-like structure.

An analysis of the potentiometric data (Table 1) showed that the addition of honey to the hydrogel composition contributed to a decrease in the pH value. It is known that depending on the skin type, the pH can vary 4–7. Since our results fall within the permissible range, honey-containing hydrogels can be considered suitable for transdermal administration.

The viscosity parameters of the samples also depended on the incorporation of honey into the hydrogel composition (Table 2). It was observed that viscosity decreased with an increase in honey concentration in the hydrogels. This trend was characteristic of all samples, except for those based on high-viscosity chitosan. The most suitable parameters for transdermal administration were exhibited

by samples based on Carbopol 940 with a 25 wt % honey concentration.

To determine the sorption capacity of hydrogels, the moisture content of each sample was calculated in mass fractions using formula (2). The results of the moisture content calculations are presented in Table 3.

The data obtained on the sorption capacity of the hydrogels are presented in Table 4. Samples with the lowest honey concentration demonstrated the highest sorption capacity, regardless of the type of polymer they were based on. Observations were conducted over 1 h and 3 h. These time intervals were selected based on the anticipated exposure time of the future coating.

It is also noteworthy that the sorption capacity of chitosan-based hydrogels decreased after 3 h. This is associated with the relatively rapid release of the substance into the buffer medium observed in chitosan-based samples. This trend was clearly demonstrated in the release test. The opposite trend was observed in carbopol-based samples.

The greatest sorption performance was demonstrated by the sample based on low-viscosity chitosan with a 25 wt % honey concentration, both after 1 h and 3 h. This sample yielded the sorption capacity values of 14.63 g/g. Furthermore, no significant decrease in sorption capacity was observed after 3 h. The lowest performance levels were observed in the sample based on high-viscosity chitosan with a 50 wt % honey concentration. This sample exhibited a low sorption capacity of 2.74 g/g after 1 h of the experiment, and after 3 h, the sorption capacity decreased more than fivefold down to 0.53 g/g.

Table 1. Results of potentiometric study*

Polymer name	Honey concentration, wt %	pH values of polymer solutions after adjustment with triethanolamine	pH values of hydrogels after honey addition
3.5% High-viscosity chitosan	25	6.5	6.4
	50		6.2
	75		6.3
3.5% Low-viscosity chitosan	25	6.3	6.2
	50		6.3
	75		5.3
1% Carbopol 940	25	6.3	6
	50		5.8
	75		5.7
1% Carbopol ETD 2020	25	6.3	5.9
	50		5.8
	75		5.7

Table compiled by the authors based on original data

Note: * the table presents average values.

Table 2. Results of hydrogel viscosity studies*

Polymer name	Honey concentration, wt %	Viscosity parameters of polymer solutions, cPs	Viscosity parameters of hydrogels after honey addition, cPs
3,5% High-viscosity chitosan	25	9.2	7.2
	50		4.9
	75		6.8
3,5% Low-viscosity chitosan	25	13.28	4.24
	50		4.3
	75		1.98
1% Carbopol 940	25	25.68	10.96
	50		5.6
	75		1.2
1% Carbopol ETD 2020	25	18.08	8
	50		5.12
	75		1.1

Table compiled by the authors based on original data
Note: * the table presents average values; cPs — centipoise.

Table 3. Assessment of hydrogel swelling capacity*

Polymer name	Honey concentration, wt %	Moisture content, %	Standard deviation (SD)
3.5% High-viscosity chitosan	25	0.63	0.03
	50	0.65	0.02
3.5% Low-viscosity chitosan	25	0.63	0.04
	50	0.68	0.03
1% Carbopol 940	25	0.67	0.06
	50	0.67	0.07
1% Carbopol ETD 2020	25	0.61	0.05
	50	0.60	0.06

Table compiled by the authors based on original data
Note: * the table presents average values.

The spreadability study of the samples, including the standard deviation (SD), are presented in Table 5. It was found that an increase in honey concentration was associated with smaller droplet diameters, indicating reduced hydrogel spreadability. The comparison study revealed that the low-viscosity chitosan-based samples exhibited the highest spreadability. The smallest spreadability value of 0.55 cm was observed for samples based on Carbopol ETD 2020 with a 50 wt % honey concentration, as well as for samples based on high-viscosity chitosan with a 50 wt % honey concentration, making them more preferable for use as a transdermal coating.

These data correlate with the results previously obtained by other authors [19]. Future research should investigate the spreadability of a hybrid hydrogel composed of Carbopol ETD 2020 and low-viscosity chitosan. Presumably, such a hydrogel would distribute better over the wound surface without spreading beyond its boundaries.

The stability study of the hydrogels revealed that all samples, except for those with a 75 wt % honey concentration, passed the test successfully. No precipitate formation or phase separation was detected after centrifugation.

Table 4. Results of sorption capacity studies*

Polymer name	Honey concentration, wt %	Sorption capacity after 1 h	Standard deviation (SD)	Sorption capacity after 3 h	Standard deviation (SD)
3.5% High-viscosity chitosan	25	10.44	0.09	7.24	0.05
	50	2.74	0.08	0.53	0.07
3.5% Low-viscosity chitosan	25	14.63	0.13	14.37	0.11
	50	5.34	0.08	1.23	0.02
1% Carbopol 940	25	7.33	0.06	10.00	0.04
	50	3.16	0.11	3.50	0.06
1% Carbopol ETD 2020	25	4.71	0.07	6.50	0.09
	50	2.57	0.03	4.54	0.03

Table compiled by the authors based on original data

Note: * the table presents average values.

Table 5. Results of spreadability studies

Polymer name	Honey concentration, wt %	Mean value, cm	Standard deviation (SD)
3.5% High-viscosity chitosan	25%	1.03	0.09
	50%	0.76	0.07
3.5% Low-viscosity chitosan	25%	1.66	0.13
	50%	1.36	0.06
1% Carbopol 940	25%	1.00	0.09
	50%	0.93	0.12
1% Carbopol ETD 2020	25%	1.01	0.06
	50%	0.55	0.11

Table compiled by the authors based on original data

For the study of honey release from hydrogels, samples based on 10% high-viscosity chitosan and 10% low-viscosity chitosan were prepared instead of the 3.5% samples. The sample based on low-viscosity chitosan with a 50 wt % honey concentration demonstrated the best honey release profile. This sample showed the highest rate of honey release as early as the first hour of the experiment. Subsequently, the release values continued to increase. For the other samples, only a slight increase in the concentration of released honey was characteristic. The study results are presented in Figure 4.

Different varieties of floral honey exhibit pH values ranging 3.5–4.1. However, linden honey with pH varying 4.5–7 is an exception. A neutral or slightly alkaline environment is most favorable for the comfortable survival of most bacteria. If the environmental pH shifts strongly to the alkaline region (pH 8–14), cellular RNA and phospholipids are destroyed, leading to cell death.

Conversely, when the environmental pH shifts to the acidic region (pH 1–6), DNA and ATP are degraded. An acidic environment is particularly detrimental to putrefactive bacteria.

The *in vitro* study demonstrated that samples based on both low-viscosity and high-viscosity chitosan, regardless of honey concentration, exhibited an antibacterial effect against *Staphylococcus aureus* bacteria (Fig. 5).

Furthermore, samples based on both low- and high-viscosity chitosan with a 25 wt % honey concentration exhibited antibacterial properties against *Acinetobacter baumannii* bacteria (Fig. 6).

The study also identified bacteriostatic properties — the ability to suppress the growth and development — in samples based on low-viscosity chitosan with a 25 wt % honey concentration, as well as in samples based on Carbopol ETD 2020 with a 25 wt % honey concentration (Fig. 7), against *Klebsiella pneumoniae* bacteria.

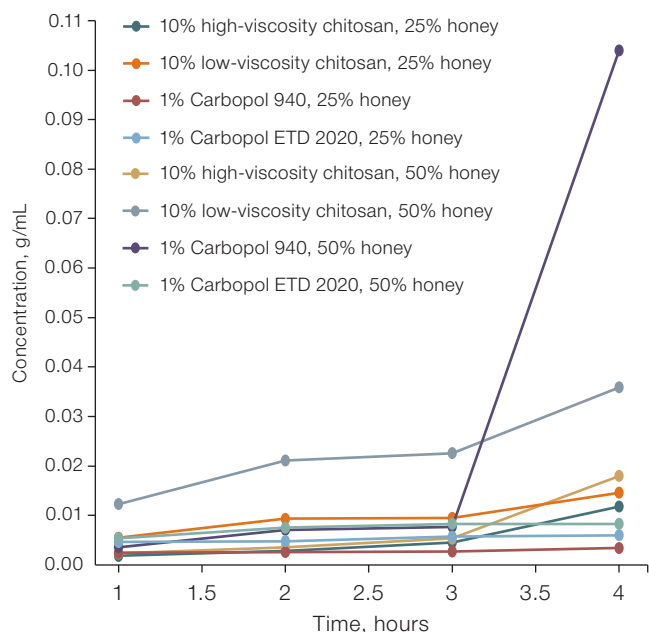
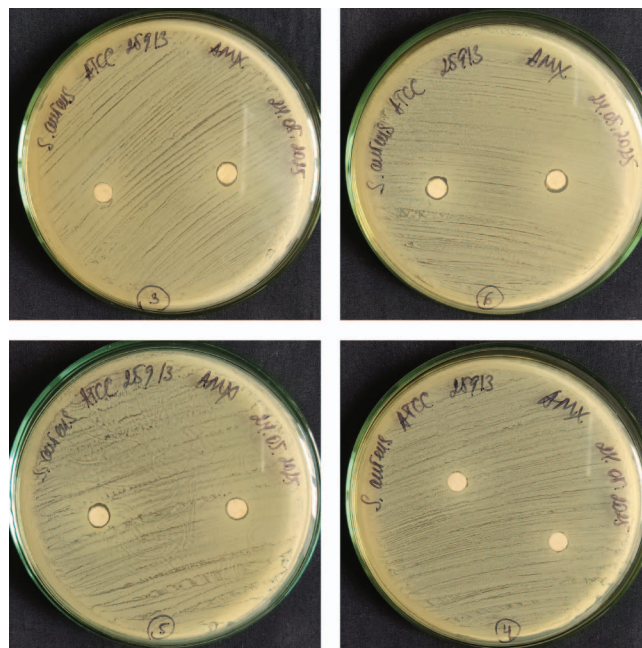


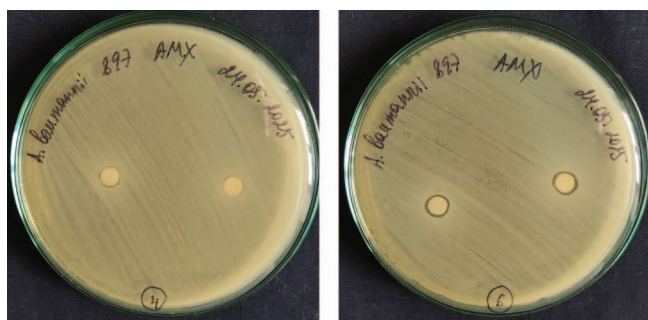
Figure prepared by the authors using their own data

Fig. 4. Results of honey release from hydrogels



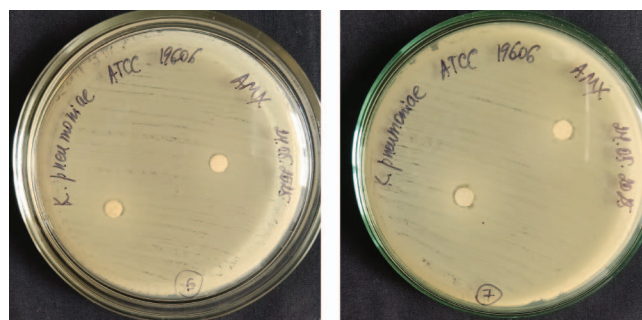
Photos taken by the authors

Fig. 5. Study of antibacterial properties of chitosan-based hydrogels against *Staphylococcus aureus*



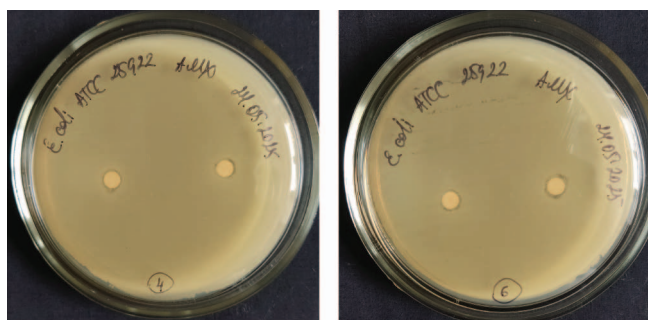
Photos taken by the authors

Fig. 6. Study of antibacterial properties of chitosan- and 25% honey-based hydrogels against *Acinetobacter baumannii*



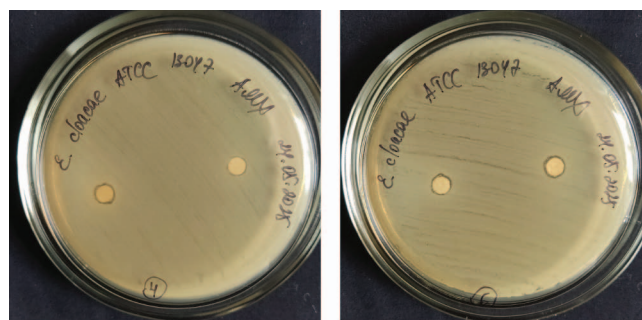
Photos taken by the authors

Fig. 7. Study of antibacterial properties of low-viscosity chitosan- and 25% honey-based hydrogels, as well as Carbopol ETD 2020-based hydrogel with 25% honey concentration, against *Klebsiella pneumoniae*



Photos taken by the authors

Fig. 8. Study of antibacterial properties of chitosan- and 25% honey-based hydrogels against *Escherichia coli*



Photos taken by the authors

Fig. 9. Study of antibacterial properties of chitosan- and 25% honey-based hydrogels against *Enterobacter cloacae*

Furthermore, a weak bacteriostatic effect was observed from hydrogels based on both low- and high-viscosity chitosan with a 25 wt % honey concentration against *Escherichia coli* (Fig. 8) and *Enterobacter cloacae* (Fig. 9).

The remaining samples did not exhibit antibacterial or bacteriostatic properties, which may be associated with weak honey release. Furthermore, no antifungal effect was detected from the samples, as tests against the yeast-like fungus *Candida albicans* yielded no results. This is likely due to the optimal pH for *Candida albicans* viability being 6.0–6.8.

However, it is important to note that the effects observed were demonstrated against “wild-type” strains of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*. In the future, we plan to conduct a series of experiments on polyresistant hospital-acquired and community-acquired strains of the Gram-negative pathogens *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*.

CONCLUSIONS

1. The hydrogel based on 1% Carbopol 940 with a 25 wt % honey concentration outperformed other samples in terms of viscosity. The hydrogel based on 3.5% low-viscosity chitosan showed the lowest viscosity value.

2. The samples based on 1% Carbopol ETD 2020 with a 50% honey concentration exhibit the lowest

spreadability. The highest spreadability was noted for 3.5% low-viscosity chitosan, making it an undesirable candidate as a transdermal coating base.

3. To achieve a coating with optimal viscosity characteristics and spreadability, it is recommended to further investigate a mixed hydrogel composed of Carbopol 940 and low-viscosity chitosan.

4. The greatest sorption capacity after 1 h and 3 h from the start of the test was demonstrated by the sample based on 3.5% low-viscosity chitosan with a 25% honey concentration.

5. The highest honey release was demonstrated by the sample based on 10% low-viscosity chitosan with a 50% honey concentration. The sample based on 3.5% low-viscosity and high-viscosity chitosan proved unsuitable for further research.

6. According to the results of antibacterial action studies, chitosan-based hydrogels were identified as the most promising samples for further investigation.

7. The conducted study of the physicochemical parameters of the investigated hydrogels and their antibacterial properties allow us to recommend a mixed hydrogel based on Carbopol 940 and 3.5% low-viscosity chitosan for further investigation.

8. The comparative analysis revealed hydrogel samples with a 75 wt % honey concentration to fail the stability test, making them unsuitable for use as a therapeutic delivery system.

References

- Bibire T, Panainte AD, Yilmaz CN, Timofte DV, Danila R, Bibire N, et al. Dexketoprofen-Loaded Alginate-Grafted Poly(N-vinylcaprolactam)-Based Hydrogel for Wound Healing. *International Journal of Molecular Sciences*. 2025;26(7):3051. <https://doi.org/10.3390/ijms26073051>
- Surowiecka A, Struzyna J, Winiarska A, Korzeniowski T. Hydrogels in Burn Wound Management—A Review. *Gels*. 2022;8:122. <https://doi.org/10.3390/gels8020122>
- Yao Y, Zhang A, Yuan C, Chen X, Liu Y. Recent trends on burn wound care: Hydrogel dressings and scaffolds. *Biomaterials Science*. 2021;9(13):4523–40. <https://doi.org/10.1039/d1bm00411e>
- Chornopyschuk R, Nagaichuk V, Gerashchenko I, Nazarchuk H, Kukolevska O, Chornopyschuk N, et al. Antimicrobial properties of a new polymeric material based on poly (2-hydroxyethyl methacrylate). *Acta Bio-Medica: Atenei Parmensis*. 2022;93(1):e2022012.
- Romero-Antolin JA, Gomez-Cerezo N, Manzano M, Pablos JL, Valet-Regi M. Anti-inflammatory and Antibacterial Hydrogel Based on a Polymerizable Ionic Liquid. *Acta Biomaterialia*. 2025;196:78–92. <https://doi.org/10.1016/j.actbio.2025.03.015>
- Maikovich O, Nosova N, Bukartuk N, Fihurka N, Ostapiv D, Samaryk V, et al. Gelatin-based hydrogel with antiseptic properties: synthesis and properties. *Applied Nanoscience*. 2023;13(12):7611–23. <https://doi.org/10.1007/s13204-023-02956-6>
- Amanzholkzy A, Zhumagaliyeva S, Sultanova N, Abilov Z, Ongalbek D, Donbayeva E, et al. Hydrogel Delivery System for Biological Active Substances: Properties and the Role of HPMC as a Carrier. *Molecules*. 2025;30(6):1354. <https://doi.org/10.3390/molecules30061354>
- Li M, Qu H, Li Q, Lu S, Wu Y, Tang Z, et al. A carboxymethyl cellulose/chitosan-based hydrogel harvests robust adhesive, on-demand detachment and self-healing performances for deep burn healing. *Chemical Engineering Journal*. 2024;498:155552. <https://doi.org/10.1016/j.cej.2024.155552>
- Nweze JA, Olovo CE, Nweze EI, Okechukwu OJ, Chidebelu P. Therapeutic Properties of Honey. In: de Toledo VA, Chambo ED, editors. *Honey Analysis: New Advances and Challenges*. London: IntechOpen; 2020:17–39. <https://doi.org/10.5772/intechopen.86416>
- Albaridi NA. Antibacterial Potency of Honey. *International Journal of Microbiology*. 2019:1–10. <https://doi.org/10.1155/2019/2464507>
- Sayadi SA, Zohdi RM, Shamshuddin NSS, Khairy MS, Hasan NA, Yasin AS, et al. Antifungal activity of selected Malaysian honeys: a comparison with Manuka honey. *Journal of Coastal Life Medicine*. 2015;3(7):539–42.
- Francois AE, Gbaguidi B, Paraiso AA, Dah-Nouvlessounon D, Goubalan E, Baba-Moussa F, et al. Polyphenolic profile, and antioxidant and antifungal activities of honey products in Benin. *African Journal of Microbiology Research*. 2018;12(1):9–18. <https://doi.org/10.5897/AJMR2017.8749>

13. Khan RU, Naz S, Abudabos AM. Towards a better understanding of the therapeutic applications and corresponding mechanisms of action of honey. *Environmental Science and Pollution Research*. 2017;24(36):27755–66. <https://doi.org/10.1007/s11356-017-0567-0>
14. Talebi M, Talebi M, Farkhondeh T, Samarghandian S. Molecular mechanism-based therapeutic properties of honey. *Biomedicine & Pharmacotherapy*. 2020;130:110590. <https://doi.org/10.1016/j.biopha.2020.110590>
15. Tashkandi H. Honey in wound healing: An updated review. *Open Life Sciences*. 2021;16(1):1091–100. <https://doi.org/10.1515/biol-2021-0084>
16. Bahari N, Hashim N, Akim AM, Maringgal B. Recent Advances in Honey-Based Nanoparticles for Wound Dressing: A Review. *Nanomaterials*. 2022;12(15):2560. <https://doi.org/10.3390/nano12152560>
17. Molan P.C. Potential of Honey in the Treatment of Wounds and Burns. *American Journal of Clinical Dermatology*. 2001;2(1):13–9. <https://doi.org/10.2165/00128071-200102010-00003>
18. El-Kased RF, Amer RI, Attia D, Elmazar MM. Honey-Based Hydrogel: In Vitro and In Vivo Evaluation for Burn Wound Healing. *Scientific Reports*. 2017;7:9692. <https://doi.org/10.1038/s41598-017-08771-8>

Authors' contributions. All authors confirm that their contributions meet the ICMJE authorship criteria. The primary contributions are distributed as follows: Ekaterina N. Karpina — sample preparation, investigation of their physicochemical properties, interpretation of the obtained results; Elena V. Fedotova — oversight during research planning and execution, data collection and analysis for the work, writing the initial draft of the manuscript, creation and preparation of the manuscript; Faliya F. Zaripova — investigation of the *in vitro* antibacterial properties of the samples, critical analysis of the manuscript draft, providing comments and revisions; Denis V. Krivorotov — data collection and analysis for the work, critical analysis of the work for scientific novelty; Andrey S. Radilov — formulation of the research objectives, provision of access to necessary research materials, reagents, substances, and laboratory samples, research design, final approval of the version for publication.

AUTHORS

Ekaterina N. Karpina

<https://orcid.org/0009-0008-1811-2453>
karpinaekaterina@gmail.com

Elena V. Fedotova, Cand. Sci. (Chem.)

<https://orcid.org/0000-0001-6056-1983>
arabka2008@mail.ru

Faliya F. Zaripova, Cand. Sci. (Biol.)

<https://orcid.org/0000-0002-4196-1882>
faliabio@yandex.ru

Denis V. Krivorotov, Cand. Sci. (Chem.)

<https://orcid.org/0000-0002-6077-2534>
denhome@bk.ru

Andrey S. Radilov, Dr. Sci. (Med.), Professor

<https://orcid.org/0000-0003-0776-7434>
a.radilov@icloud.com



Scientific and practical reviewed journal
of FMBA of Russia
extrememedicine.ru