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CLONAL HEMATOPOIESIS WITH INDETERMINATE POTENTIAL IN TET2 AND DNMT3A AMONG KAZAKHSTANI INDIVIDUALS WITH ATHEROSCLEROTIC DISEASE

Makhabbat S. Bekbossynova[1], https://orcid.org/0000-0003-2834-617X
Tomiris K. Shakhmarova[2], https://orcid.org/0009-0008-6884-3908
Zhanel Zh. Mirmanova [2], https://orcid.org/0000-0002-0284-3891
Madina R. Zhalbinova[2], https://orcid.org/0000-0001-9704-8913
Ayaulym E. Chamoieva[2], https://orcid.org/0000-0003-0877-3537
Nazerke N. Satvaldina[2], https://orcid.org/0000-0002-0611-4485
Saule E. Rakhimova[2], https://orcid.org/0000-0002-8245-2400
Dauren A. Yerezhepov[2], https://orcid.org/0000-0002-4161-1348
Ulykbek E. Kairov[2], https://orcid.org/0000-0001-8511-8064
Asset Zh. Daniyarov [2], https://orcid.org/0000-0003-3886-718X
Saltanat A. Andossova[1], https://orcid.org/0000-0001-7259-183X
Tatyana V. Ivanova-Razumova[1], https://orcid.org/0000-0002-6471-1587
Aliya I. Sailybayeva[1], https://orcid.org/0000-0002-1489-3837
Sadyk R. Khamitov[1], https://orcid.org/0000-0001-6296-0507
Ainur R. Akilzhanova[2,3,4], https://orcid.org/0000-0001-6161-8355

[1] University Medical Center Corporate Fund, Heart Center, Nazarbayev University, Astana, Republic of Kazakhstan;
[2] Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana, Republic of Kazakhstan;
[3] Eurasian Society of Personalized Medicine, Astana, Republic of Kazakhstan;
[4] The Academy of Public Administration under the President of the Republic of Kazakhstan, Astana, Republic of Kazakhstan.

Abstract

Purpose of the study: To assess the contribution of clonal hematopoiesis of indeterminate potential (CHIP) to the development of atherosclerotic disease in the Kazakhstani cohort, focusing on the *TET2* and *DNMT3A* genes, based on next-generation sequencing (NGS) data.

Materials and Methods: This study employed an observational cross-sectional design. The cohort comprised 177 women and 225 men, with a mean age of 53.0 ± 9.04 years overall (men: 52.2 ± 9.0 years; women: 53.7 ± 9.0 years). Patients were stratified cardiovascular risk category (low, high, very high) according to the ESC/EAS (2019) recommendations. In this sample, 74 participants (18.4%) were classified as low-risk, 136 (33.8%) as high-risk, and 192 (47.8%) as very high-risk. For the initial phase of analysis, the two most studied genes, *TET2* and *DNMT3A*, were selected from a total list of 8 priority genes: *ASXL1, DNMT3A, JAK2, PPM1D, SF3B1, SFRS2, TET2,* and *TP53* associated with CHIP, based on their high clinical significance and frequency of occurrence in previously published studies. By filtering the data and selecting two priority genes for primary analysis, 41 patients with atherosclerotic disease were identified from the total cohort. Genetic analyses were performed using high-throughput whole-exome sequencing to cover a wide range of genetic variants potentially associated with CHIP and the atherosclerotic process. Variant annotation was performed using international databases (ClinVar, ExAC, 1000 Genomes, etc.), and mutations were interpreted according to the ACMG/AMP classification using the InterVar platform. Statistical data processing included the nonparametric Kruskal-Wallis test, Dunn's test for pairwise comparisons, and Fisher's exact test; a value of $p < 0.05$ was considered statistically significant.

Results: Variants were detected in *TET2* (n=35) and *DNMT3A* (n=6). The median VAF for TET2 was 50%. Age differed significantly between risk groups (p = 0.026). Most detected variants had an ultra-rare frequency according to international databases (<0.1%). One pathogenic variant was identified according to the InterVar classification; the others were predominantly of uncertain significance (VUS). The distribution of variants by risk groups showed no statistically significant differences ($p = 0.341$).

Conclusion: The results demonstrate a potential role of CHIP-associated mutations in atherosclerosis pathogenesis, with*TET2* mutations appearing most prominent. Given the identified genetic features and their association with

cardiovascular risk, the findings justify extending the analysis to the full spectrum of CHIP-associated genes to improve the accuracy of risk stratification.

Keywords: *clonal hematopoiesis of indeterminate potential, atherosclerosis, cardiovascular disease, coronary artery disease.*

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Резюме

КЛОНАЛЬНЫЙ ГЕМОПОЭЗ НЕОПРЕДЕЛЁННОГО ПОТЕНЦИАЛА В ГЕНАХ TET2 И DNMT3A У ПАЦИЕНТОВ С АТЕРОСКЛЕРОТИЧЕСКОЙ БОЛЕЗНЬЮ В КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ

Махаббат С. Бекбосынова[1], https://orcid.org/0000-0003-2834-617X
Томирис К. Шахмарова[2], https://orcid.org/0009-0008-6884-3908
Жанель Ж. Мирманова[2], https://orcid.org/0000-0002-0284-3891
Мадина Р. Жалбинова[2], https://orcid.org/0000-0001-9704-8913
Аяулым Е. Чамойева[2], https://orcid.org/0000-0003-0877-3537
Назерке Н. Сатвалдина[2], https://orcid.org/0000-0002-0611-4485
Сауле Е. Рахимова[2], https://orcid.org/0000-0002-8245-2400
Даурен А. Ережепов[2], https://orcid.org/0000-0002-4161-1348
Улыкбек Е. Каиров[2], https://orcid.org/0000-0001-8511-8064
Асет Ж. Данияров[2], https://orcid.org/0000-0003-3886-718X
Салтанат А. Андосова[1], https://orcid.org/0000-0001-7259-183X
Татьяна В. Иванова-Разумова[1], https://orcid.org/0000-0002-6471-1587
Алия И. Сайлыбаева[1], https://orcid.org/0000-0002-1489-3837
Садык Р. Хамитов[1], https://orcid.org/0000-0001-6296-0507
Айнур Р. Акильжанова[2,3,4.], https://orcid.org/0000-0001-6161-8355

[1] Корпоративный фонд «University Medical Center», Центр сердца, Назарбаев Университет, г. Астана, Республика Казахстан;
[2] Центр наук о жизни, National Laboratory Astana, Назарбаев Университет, г. Астана, Республика Казахстан;
[3] Евразийское Общество Персонализированной Медицины, г. Астана, Республика Казахстан;
[4] Академия государственного управления при Президенте Республики Казахстан, г. Астана, Республика Казахстан.

Цель исследования: Оценить вклад клонального гемопоэза неопределенного потенциала (КГНП) в развитие атеросклеротического заболевания в казахстанской когорте с упором на гены *TET2* и *DNMT3A* на основе данных секвенирования нового поколения (NGS).

Материалы и методы: Проведено обсервационное поперечное исследование. Когорта состояла из 177 женщин и 225 мужчин, средний возраст которых в целом составил 53,0 ± 9,04 года (мужчины: 52,2 ± 9,0 года; женщины: 53,7 ± 9,0 года). Пациенты были распределены по категориям сердечно-сосудистого риска (низкий, высокий, очень высокий) в соответствии с рекомендациями ESC/EAS (2019). В этой выборке 74 участника (18,4%) были отнесены к группе низкого риска, 136 (33,8%) – к группе высокого риска и 192 (47,8%) – к группе очень высокого риска. Для начальной фазы анализа два наиболее изученных гена, *TET2* и *DNMT3A*, были выбраны из общего списка восьми приоритетных генов: *ASXL1, DNMT3A, JAK2, PPM1D, SF3B1, SFRS2, TET2* и *TP53*, ассоциированных с КГНП, на основании их высокой клинической значимости и частоты в ранее опубликованных исследованиях. Путем фильтрации данных и выбора двух приоритетных генов для первичного анализа из общей когорты были выделены 41 пациент с атеросклеротическим заболеванием. Генетический анализ был проведен с использованием высокопроизводительного секвенирования всего экзома (NGS, whole-exome sequencing), чтобы охватить широкий спектр генетических вариантов, потенциально связанных с КГНП и атеросклеротическим процессом. Аннотирование вариантов проводилось с использованием международных баз данных (ClinVar, ExAC, 1000 геномов и др.), а мутации интерпретировались в соответствии с классификацией ACMG/AMP с использованием платформы InterVar. Статистическая обработка данных включала непараметрический критерий Крускала-Уоллиса, критерий Данна для парных сравнений и точный критерий Фишера; значение $p < 0,05$ считалось статистически значимым.

Результаты: Варианты были обнаружены в *TET2* (n=35) и *DNMT3A* (n=6). Медиана VAF для *TET2* составила 50%. Возраст пациентов значительно различался между группами риска (p = 0,026). Большинство обнаруженных вариантов имели крайне редкую частоту согласно международным базам данных (<0,1%). Один патогенный вариант был идентифицирован в соответствии с классификацией InterVar; остальные - преимущественно варианты неопределенного значения (VUS). Распределение вариантов по группам риска не показало статистически значимых различий (p = 0,341).

Выводы: Результаты указывают на потенциальную роль CHIP-ассоциированных мутаций в патогенезе атеросклероза; наибольшее значение показали мутации *TET2*. Учитывая выявленные генетические особенности и их связь с сердечно-сосудистыми рисками, полученные данные обосновывают необходимость расширения анализа на весь спектр КГНП-ассоциированных генов для повышения точности стратификации риска.

Ключевые слова: *клональный гемопоэз с неопределенным потенциалом, атеросклероз, сердечно-сосудистые заболевания, ишемическая болезнь сердца.*

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Түйіндеме

ҚАЗАҚСТАНДЫҚ ПОПУЛЯЦИЯДАҒЫ АТЕРОСКЛЕРОТИКАЛЫҚ АУРУЫ БАР ПАЦИЕНТТЕРДЕГІ TEST2 ЖӘНЕ DNMT3A ГЕНДЕРІНДЕГІ БЕЛГІСІЗ ПОТЕНЦИАЛЫ КЛОНДЫ ГЕМОПОЭЗ

Махаббат С. Бекбосынова[1], https://orcid.org/0000-0003-2834-617X
Томирис К. Шахмарова[2], https://orcid.org/0009-0008-6884-3908
Жанель Ж. Мирманова[2], https://orcid.org/0000-0002-0284-3891
Мадина Р. Жалбинова[2], https://orcid.org/0000-0001-9704-8913
Аяулым Е. Чамойева[2], https://orcid.org/0000-0003-0877-3537
Назерке Н. Сатвалдина[2], https://orcid.org/0000-0002-0611-4485
Сауле Е. Рахимова[2], https://orcid.org/0000-0002-8245-2400
Даурен А. Ережепов[2], https://orcid.org/0000-0002-4161-1348
Улыкбек Е. Каиров[2], https://orcid.org/0000-0001-8511-8064
Асет Ж. Данияров[2], https://orcid.org/0000-0003-3886-718X
Салтанат А. Андосова[1], https://orcid.org/0000-0001-7259-183X
Татьяна В. Иванова-Разумова[1], https://orcid.org/0000-0002-6471-1587
Алия И. Сайлыбаева[1], https://orcid.org/0000-0002-1489-3837
Садык Р. Хамитов[1], https://orcid.org/0000-0001-6296-0507
Айнур Р. Акильжанова[2,3,4], https://orcid.org/0000-0001-6161-8355

[1] «University Medical Center» корпоративтік қоры, Жүрек орталығы, Назарбаев Университет, Астана қ., Қазақстан Республикасы;
[2] Өмір туралы ғылымдар орталығы, National Laboratory Astana, Назарбаев Университет, Астана қ., Қазақстан Республикасы;
[3] Еуразиялық Дербес Медицина Қоғамы, Астана қ., Қазақстан Республикасы;
[4] Қазақстан Республикасы Президентінің жанындағы Мемлекеттік басқару академиясы, Астана қ., Қазақстан Республикасы..

Зерттеудің мақсаты: *TET2* және *DNMT3A* гендеріне баса назар аудара отырып, Қазақстандық пациенттерде атеросклеротикалық аурудың дамуына анықталмаған потенциалды клондық гемопоэздің (CHIP) үлесін жаңа буынның секвенирлеу деректері (NGS) негізінде бағалау.

Зерттеудің әдістері: Көлденең (бірмезеттік) зерттеу жүргізілді. Когортқа 177 әйел мен 225 ер адам кірді, олардың орташа жасы жалпы алғанда 53,0 ± 9,04 жасты құрады (ерлер: 52,2 ± 9,0 жас; әйелдер: 53,7 ± 9,0 жас). Пациенттер ESC/EAS (2019) нұсқауларына сәйкес жүрек-қан тамырлары қаупі бойынша (төмен, жоғары, өте жоғары) стратификацияланған. Бұл үлгіде 74 қатысушы (18,4%) тәуекелі төмен, 136 (33,8%) тәуекелі жоғары және 192 (47,8%) өте жоғары тәуекел санатына жатқызылды.Талдаудың бастапқы кезеңі үшін 8 геннің жалпы тізімінен: *ASXL1, DNMT3A, JAK2, PPM1D, SF3B1, SFRS2, TET2, TP53*, белгісіз потенциалы клондық гемопоэзбен байланысты, ең көп зерттелген екі ген

TET2, *DNMT3A* таңдалды, олардың жоғары клиникалық маңыздылығы мен бұрын жарияланған зерттеулерде пайда болу жиілігіне негізделген. Деректерді сұрыптау және бастапқы талдау үшін екі басым генді сүзгілеу нәтижесінде жалпы когортыдан атеросклеротикалық ауруы бар 41 пациент іріктелді. Генетикалық талдау толық экзомды секвинерлеу арқылы жүргізілді, бұл белгісіз потенциалы клонды гемопоэз және атеросклеротикалық процеспен ықтимал байланысты генетикалық нұсқалардың кең ауқымын қамтуға мүмкіндік берді. Генетикалық әдістердің аннотациясы халықаралық дерекқорларды (ClinVar, ExAC, 1000 Genomes және т.б.) пайдалана отырып орындалды және мутацияларды InterVar платформасын пайдалана отырып, ACMG/AMP классификациясына сәйкес түсіндіру жүргізілді. Статистикалық деректерді өңдеуге параметрлік емес Краскел-Уоллис критерийі, жұптық салыстыру үшін Данн критерийі және Фишер критерийі кірді; $p < 0.05$ мәні статистикалық маңызды болып саналды.

Нәтижелер: *TET2* (n=35), *DNMT3A* (n=6) генетикалық варианттар табылды. *TET2* үшін VAF медианасы 50% құрады. Пациенттердің жасы тәуекел топтары арасында айтарлықтай өзгерді ($p = 0.026$). Табылған нұсқалардың көпшілігі халықаралық базаларға сәйкес өте сирек жиілікке ие болды (<0.1%). Intervar классификациясы бойынша бір патогендік нұсқа анықталды; қалғандары - көбіне анықталмаған мәнді (VUS). Қауіп қатер топтары бойынша бөлу статистикалық маңызды айырмашылықтарды көрсетпеді ($p = 0.341$).

Қорытынды: Атеросклероз патогенезіндегі CHIP-пен байланысты мутациялардың әлеуетті рөлін көрсетеді; ең маңызды *TET2* мутациялары. Анықталған генетикалық ерекшеліктерді және олардың жүрек-қан тамырлары қаупімен байланысын ескере отырып, нәтижелер тәуекелді стратификациялаудың дәлдігін арттыру үшін CHIP байланысты гендердің барлық спектріне талдауды кеңейту қажеттілігін негізделеді.

Негізгі сөздер: белгісіз потенциалы клондық гемопоэз, атеросклероз, жүрек-қан тамырлары аурулары, жүректің ишемиялық ауруы.

Дәйексөз үшін:

Бекбосынова М.С., Шахмарова Т.К., Мирманова Ж.Ж., Жалбинова М.Р., Чамойева А.Е., Сатвалдина Н.Н., Рахимова С.Е., Ережепов Д.А., Каиров У.Е., Данияров А.Ж., Андосова С.А., Иванова-Разумова Т.В., Сайлыбаева А.И., Хамитов С.Р., Акильжанова А.Р. Қазақстандық Популяциядағы Атеросклеротикалық Ауруы Бар Пациенттердегі TEST2 және DNMT3A Гендеріндегі Белгісіз Потенциалы Клонды Гемопоэз (CHIP) // Ғылым және Денсаулық сақтау. 2025. Vol.27 (5), Б. 16-23. *doi 10.34689/SH.2025.27.5.002*

Introduction

Atherosclerosis continues to be one of the leading causes of premature mortality and disability across the world. Although traditional risk factors such as arterial hypertension, dyslipidemia, smoking, and diabetes are well recognised, not all cases can be explained by these factors at the time of the onset of severe cardiovascular disease. In recent years, researchers have actively explored the role of somatic mutations occurring in hematopoietic cells in the pathogenesis of atherosclerosis. This phenomenon, referred to as clonal hematopoiesis of indeterminate potential (CHIP), is an age-related process wherein somatic mutations in multiple genes promote the expansion of individual clones without exhibiting signs of hematologic malignancies [1,2].

CHIP mutations are most commonly found in genes such as *ASXL1, DNMT3A*, *TET2*, *TP53*, and *PPM1D*, among others, and have been shown in several studies to be associated with a 40-50% increased risk of cardiovascular mortality [3,4]. It is hypothesized that clonal cells with mutations in *TET2* and *DNMT3A* produce pro-inflammatory cytokines, thereby activating monocytic and macrophage cascades, which contribute to the progression of atherosclerotic plaques [5,6].

While the role of clonal hematopoiesis of uncertain potential (CHIP) in the pathogenesis of atherosclerotic disease has been thoroughly explored in recent years, most existing data come from cohorts in Europe and North America. This creates a notable gap in the global understanding of the prevalence and molecular characteristics of CHIP in other regions. The scarcity of data from Central Asian countries restricts the ability to compare and interpret findings within a global context. The present study aims to address this information gap by providing the first systematic data on the frequency and types of CHIP-associated mutations in patients with atherosclerosis in Kazakhstan.

The purpose of the study

The present study aims to improve outcomes for patients with atherosclerotic cardiovascular diseases by developing an effective early genetic screening program that enables the differentiation of risk groups based on molecular genetic data. To achieve this goal, we performed high-throughput whole-exome sequencing (WES) to detect mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and predisposition to atherosclerosis.

Research Objectives

To identify somatic variants in the *TET2* and *DNMT3A* genes among patients with atherosclerotic diseases.

To estimate the frequency of pathogenic, likely pathogenic and variants of uncertain significance (VUS) based on InterVar classification and frequency of occurrence in population databases.

To correlate molecular characteristics of variants with clinical parameters (age, gender, clinical risk).

To compare the level of clonality (VAF) between the two genes.

Materials and Methods

An observational analytical study (single-stage, cross-sectional) was conducted to investigate the frequency and molecular characteristics of mutations associated with CHIP in a cohort of young Kazakhstani individuals with atherosclerotic cardiovascular disease. DNA samples from patients (n = 402) with a confirmed diagnosis of atherosclerosis were included. Whole-exome sequencing (WES) was used to identify genetic

variants potentially associated with CHIP. Inclusion in the analysis was based on the availability of high-quality NGS data and compliance with predefined preprocessing criteria. Genomic loci with wild-type genotype were excluded, as they contained no allelic substitutions. For further analysis, only samples carrying heterozygous or homozygous variants were selected to focus on mutations with potential functional impact. Patients were stratified into cardiovascular risk categories (low, high, and very high) based on the 2019 ESC/EAS Guidelines [7]. The evaluation criteria included clinical parameters, low-density lipoprotein cholesterol (LDL-C) levels, and the presence of diabetes, hypertension, target organ damage, chronic kidney disease, and other risk factors.

Additional filtering of genetic variants was performed based on their minor allele frequency (MAF < 1%) using international databases (ExAC, 1000 Genomes, PopFreq). Variant annotation was performed using ANNOVAR and other bioinformatics tools, and clinical interpretation was based on the ACMG/AMP guidelines via the InterVar platform. In our initial analysis, we examined two of the eight genes associated with the development of CHIP specifically: *ASXL1, DNMT3A, JAK2, PPM1D, SF3B1, SFRS2, TET2,* and *TP53*. At this stage, *TET2* and *DNMT3A* were selected for a more in-depth analysis; the remaining genes will be analyzed in subsequent work. Sequencing data annotated using the following software tools were used for analysis: ANNOVAR, GATK, BWA, Bowtie/Bowtie2, VarScan. Annotation and functional interpretation of variants were performed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP). Mutations were classified on the InterVar platform into five categories: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), and benign (B).

To quantify clonality, we utilised variant allele frequency (VAF), which is calculated as the ratio of reads containing a variant to the total number of reads at a specific position. VAF values were expressed as percentages and subsequently converted to decimal proportions for statistical analysis. Only variants with a VAF ≥ 2% were analysed, in accordance with standard thresholds for detecting clonal events. In parallel, allele frequencies in international databases, such as ExAC, 1000 Genomes, and PopFreq, were taken into account, with a minor allele frequency (MAF) threshold of < 0.01 to select rare variants.

Data processing and visualisation were performed in the R software (version 4.4) using the tidyverse, ggplot2, dplyr, survival, and survminer libraries. Data are presented as absolute and relative frequencies, medians, and interquartile ranges (Me [IQR]). Nonparametric methods were used to compare quantitative indices between groups, including the Kruskal-Wallis test and the Mann-Whitney test. Fisher's exact test and χ^2 test were used to assess differences in the distribution of categorical variables. The critical level of significance was set at $p < 0.05$. The p-values are presented rounded to three decimal places. All tests were two-sided.

Results

At the time of analysis, variants in 8 of the 74 selected genes involved in the pathogenesis of CHIP were fully annotated and analysed. Two genes, *TET2* and *DNMT3A*, were selected for the present analysis as the most frequently associated with CHIP mutations. After applying the filtering criteria, 41 out of 402 patients (10.2%) were identified as carriers of relevant variants. Further stratification by cardiovascular risk revealed that 14 patients were classified into the low-risk group, 10 patients into the high-risk group, and 17 patients into the very high-risk group. As a result, after stepwise filtering, a distinct subgroup of patients with CHIP was identified, representing approximately one-tenth of the total study cohort.

Kernel-density violin plots illustrate the age distribution in all risk groups (Fig. 1). The mean ± SD ages were 46.8 ± 10.4 years (low-risk), 54.2 ± 12.7 years (high-risk), and 56.9 ± 8.8 years (very high-risk). Age increased significantly with risk category (*Kruskal-Wallis* $\chi^2 = 6.61$, $p = 0.037$). Pairwise Dunn tests revealed a significant difference between the low and very high-risk groups (adjusted $p = 0.026$). This analysis aimed to determine whether age varied significantly among risk groups, as age is a known factor associated with CHIP and may influence the distribution of genetic variants within the studied cohort.

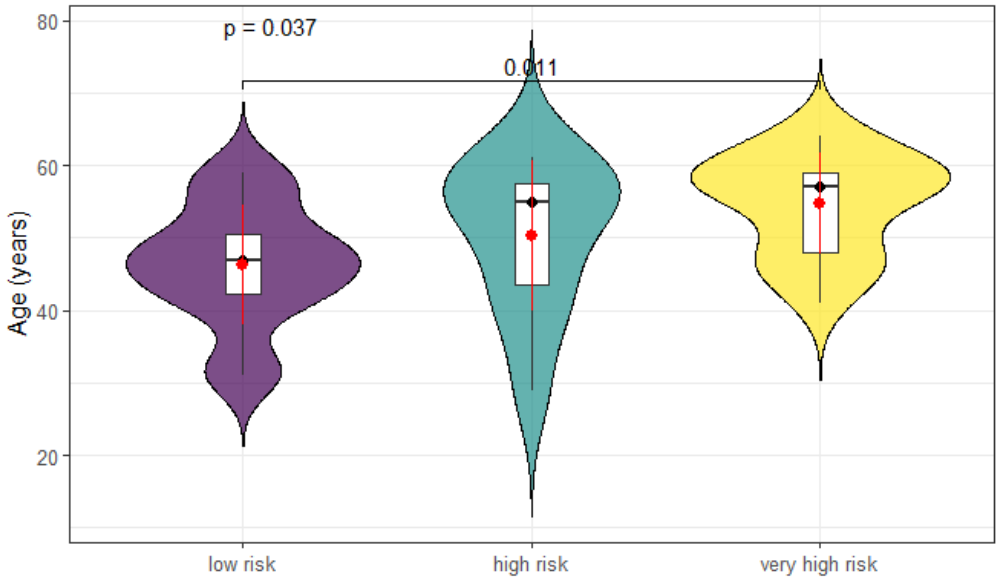

Fig. 1 Age distribution across risk categories

To assess the relationship between the type of genetic variants and the degree of cardiovascular risk, patients were divided into two groups: low-risk and a combined category of high and very-high-risk (Fig. 2). Genetic variant types were classified according to ACMG/AMP guidelines using the InterVar platform into two categories: pathogenic and VUS. VUS variants predominated in both risk categories. A small proportion of pathogenic variants were observed in the low-risk group, whereas no pathogenic variants were identified in the high and very-high-risk groups. Within the combined high and very-high-risk group, all 27 variants were classified as VUS. In contrast, the low-risk group included 13 VUS and a single pathogenic variant (represented by the purple segment), resulting in proportions of 100% versus 7%. The Fisher's exact test result (p = 0.341), displayed above the bars, indicates that this difference is not statistically significant, which is in line with the limited number of pathogenic variants identified in the current dataset. These data suggest that the type of clinical interpretation of variants in the study cohort is independent of cardiovascular risk grade.

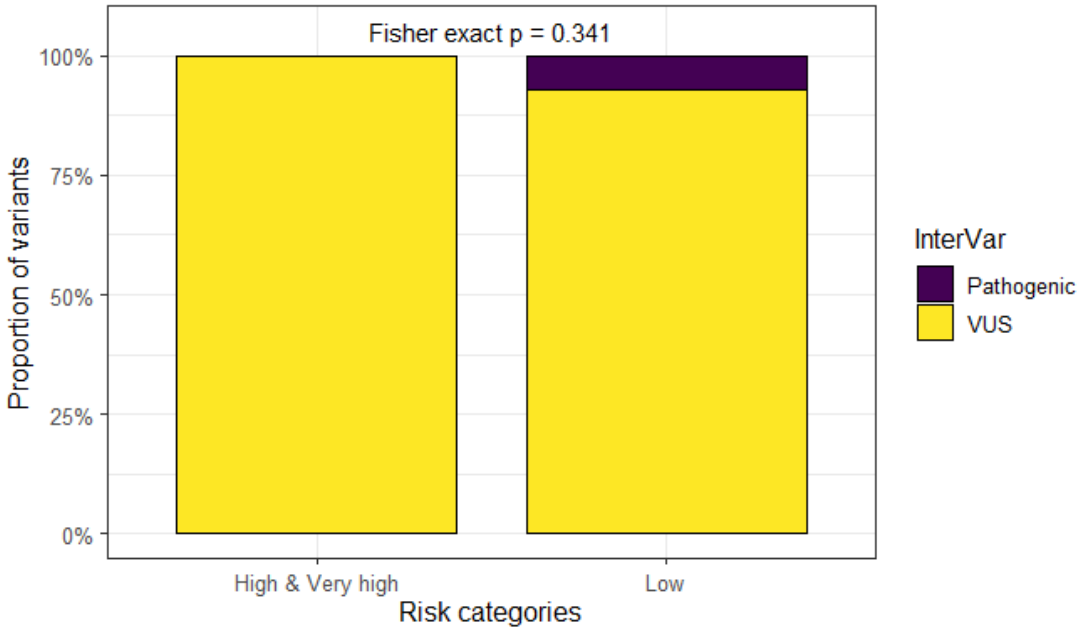

Fig. 2 Distribution of variant type across risk groups

A non-parametric Mann-Whitney rank-sum test (also known as the Wilcoxon rank-sum test) was performed to assess differences in allelic variant fraction between mutations in the *DNMT3A* (n = 6) and *TET2* (n = 35) genes. As shown in Figure 3, VAF values ranged from ~6% to ~68%, with the median value being higher for mutations in *TET2* than for those in *DNMT3A*. Statistical analysis revealed a trend towards a significant difference between the two groups (p = 0.056), which may indicate a possible difference in clone size or the timing of mutations in these genes.

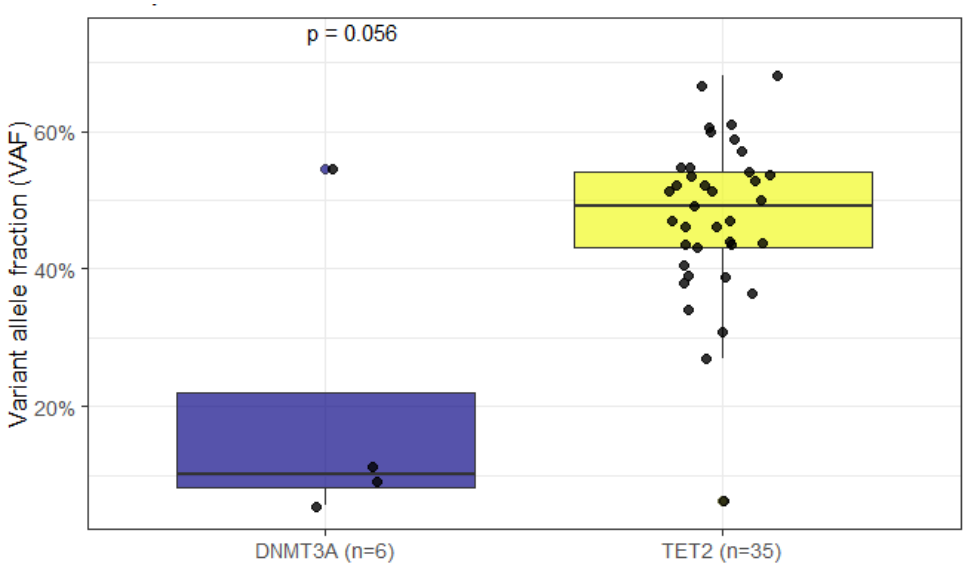

Fig. 3 VAF distribution in *DNMT3A* and *TET2*.

The bar chart (Fig. 4) illustrates the number of detected variants assigned to the MAF minor allele frequency categories: ultra-rare (< 0.1%), rare (0.1-1%), and common (≥ 1%), based on data from three population databases (ExAC, PopFreq, and 1000 Genomes ALL). In the ExAC database, the vast majority of variants identified were ultra-rare: 38 variants had MAF < 0.1%. No variants were observed in the rare category (0.1-1% in ExAC), whereas three variants were found to be frequent (MAF ≥ 1%). The PopFreq database exhibits a similar pattern: 40 variants (the majority) are ultra-rare (<0.1%), none fall within the 0.1-1% category, and only one variant reaches a frequency of> 1%. In contrast, the 1000 Genomes database (1000ALL) contains a significant proportion of variants with high frequency in the population: 27 variants (the most notable number) were classified as common (≥ 1%), and a further 14 variants fell into the rare range (0.1-1%). No ultra-rare variants (<0.1%) were identified in 1000ALL. Consequently, according to ExAC and PopFreq statistics, the majority of identified CHIP mutations are exceptionally rare or absent in the general population. In contrast, the 1000 Genomes sample includes many of these variants as common polymorphisms with a frequency of 1% or higher.

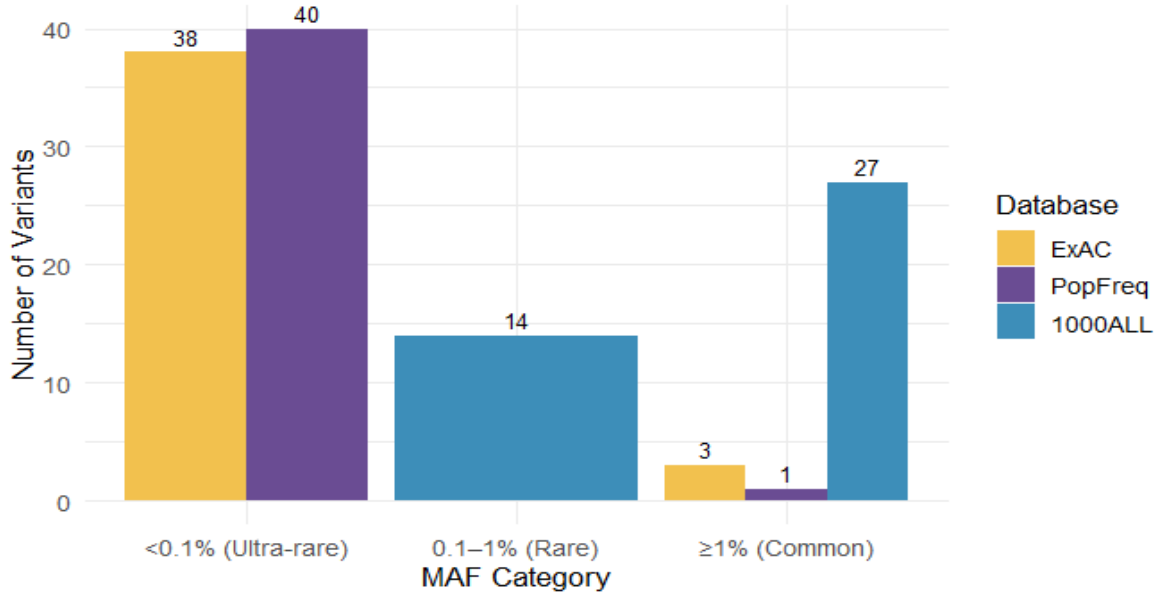

Fig.4 Distribution of MAF Across Population Datasets.

Discussion

In the present study, we analysed the frequency and molecular characteristics of CHIP in patients with atherosclerotic disease, with a particular focus on the *TET2* and *DNMT3A* genes. Data were derived from NGS analysis and stratified according to cardiovascular risk categories as outlined in the 2019 ESC/EAS guidelines [7].

One of the significant findings of the study was that mutations in *TET2* were significantly more frequent than those in *DNMT3A* (n = 35 vs. n = 6), with higher VAF values in the *TET2* group. The median VAF for *TET2* was 50%, potentially indicating the presence of mutations in larger clone progenitor cells. Although there was no statistically significant difference (p = 0.056), the results obtained may indicate differences in clonal expansion between the two genes. This finding is consistent with previous studies, which have shown that mutations in *TET2* are more frequently associated with an inflammatory cascade that promotes atherosclerosis progression [5,6].

Analysis of MAF distribution in international databases (ExAC, PopFreq, 1000ALL) showed that the majority of detected variants are ultra-rare (<0.1%), which emphasises their potential clinical significance. Ultra-rare variants are known to be more likely to have functional consequences and may be involved in the pathogenesis of chronic diseases, including cardiovascular diseases [2,3].

In addition, a comparison of variant distribution according to CVD risk revealed no statistically significant differences between groups, when classified by the InterVar classification (pathogenic and VUS variants), as confirmed by Fisher's exact test (p = 0.341). Nevertheless, almost all pathogenic variants were recorded in the low-risk group, which warrants further investigation in an expanded sample. Such ambiguity may be due to insufficient sample power or the influence of other unaccounted factors.

The age of patients was also significantly associated with the risk level: patients in the very-high-risk group were considerably older than those in the low-risk group (p = 0.026, according to Dunn's criterion), which confirms the known age dependence of CHIP [1].

Thus, our results emphasise the need to consider CHIP mutations in the context of risk assessment of atherosclerotic disease. Our data are consistent with current ideas about the role of inflammation and CHIP in the pathogenesis of CHD and justify further analysis of the broader panel of 74 genes implicated in CHIP and cardiovascular disease.

Conclusion

The present study evaluated the frequency and molecular characteristics of CHIP in patients with atherosclerosis by analysing NGS data. Of 74 genes associated with CHIP and cardiovascular disease, eight genes were selected for initial analysis, of which *TET2* and *DNMT3A* were studied in detail. Analysis revealed that mutations in *TET2* are more frequent and characterised by higher VAF values compared with *DNMT3A*, which may indicate differences in clonal expansion and pathophysiological impact on atherogenesis. Most of the identified variants were ultra-rare according to international databases, emphasising their potential significance. Despite the absence of significant differences between risk groups

according to the InterVar classification, the presence of a pathogenic variant even in the low-risk group requires attention. The results confirm the relevance of further extended analysis of all 74 genes to better assess the contribution of CHIP to atherosclerosis progression and potential risk stratification in patients.

Conflict of interests: *The authors declare no conflicts of interest.*

Contributions of authors: *All authors contributed equally to this work.*

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Author Information

Makhabbat S. Bekbossynova - Associated Professor, Deputy Chairman of the Management Board, cardiologist, “University Medical Center” Corporate Fund, Astana, Kazakhstan, Turan avenue, 38, cardiacsurgeryres@gmail.com, https://orcid.org/0000-0003-2834-617X ; 8 (7172) 70 31 00

Tomiris K. Shakhmarova - Assistant Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0009-0008-6884-3908

Zhanel Zh. Mirmanova - Assistant Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0002-0284-3891

Madina R. Zhalbinova - Junior Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan,https://orcid.org/0000-0001-9704-8913

Ayaulym E. Chamoieva - Junior Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan,https://orcid.org/0000-0003-0877-3537

Nazerke N. Satvaldina - Junior Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0002-0611-4485

Saule E. Rakhimova – Leading Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0002-8245-2400

Dauren A. Yerezhepov - Leading Researcher, Laboratory of Genomic and Personalized Medicine, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0002-4161-1348

Ulykbek E. Kairov – Head of the Laboratory of bioinformatics and systems biology, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0001-8511-8064

Asset Zh. Daniyarov - Junior Researcher, Laboratory of bioinformatics and systems biology, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0003-3886-718X

Saltanat A. Andossova - candidate of medical sciences, Chief of Cardiology #1 Department, “University Medical Center” Corporate Fund, Astana, Kazakhstan, Turan avenue, 38, https://orcid.org/0000-0001-7259-183X

Aliya I. Sailybayeva - Chief of Research Department, “University Medical Center” Corporate Fund, Astana, Kazakhstan, Turan avenue, 38, s.aliya@umc.org.kz, https://orcid.org/0000-0002-1489-3837 ; +7 701 795 17 76

Sadyk R. Khamitov - Manager of Research Department, “University Medical Center” Corporate Fund, Astana, Kazakhstan, Turan avenue, 38, https://orcid.org/0000-0001-6296-0507

***Corresponding author:**

Ainur R. Akilzhanova – Doctor of Medical Sciences, PhD, M.D., Professor, Head of Laboratory of Genomic and Personalized Medicine, Acting director, Center for Life Sciences, National Laboratory Astana, Astana, Kazakhstan, https://orcid.org/0000-0001-6161-8355

Postal address: Kabanbay batyr avenue 53, Astana, 010000, Republic of Kazakhstan;

E-mail: akilzhanova@nu.edu.kz

Phone: +7 777 658 4089 +7(7172)70-6501