# GEORGIAN MEDICAL NEWS

ISSN 1512-0112

N0 9 (330) Сентябрь 2022

ТБИЛИСИ - NEW YORK


ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

**GEORGIAN MEDICAL NEWS**

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

**GMN: Georgian Medical News** is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

**GMN: Медицинские новости Грузии -** ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

**GMN: Georgian Medical News –** საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

**WEBSITE**

www.geomednews.com

## К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

**При нарушении указанных правил статьи не рассматриваются**.

## REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.**

## ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

*GEORGIAN MEDICAL NEWS*
*No 9 (330) 2022*

*Содержание:*

*GEORGIAN MEDICAL NEWS*
*No 9 (330) 2022*

# CYP2B6 SINGLE NUCLEOTIDE POLYMORPHISMS IN AN AZERBAIJANI POPULATION

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## Abstract.

**Aim of the study:** Despite being one of the less-characterized human isoforms, cytochrome P450 2B6 is already known for its participation in the metabolism of many drugs and several environmental carcinogens. It has been studied in different populations, but ethnicity is a crucial variable to account for interindividual variability. This study aimed to investigate the genotype and allelic frequencies of CYP2B6 c.*516G>T* SNP in an Azerbaijani population, as the determination of SNP's prevalence will be helpful in further pharmacogenetics research and optimization of personalized drug therapy in Azerbaijan.

**Material and methods:** Identification of *CYP2B6*6* allelic and genotype frequencies in 100 volunteers was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The obtained results were confirmed by next-generation sequencing.

**Results:** The frequency of the **6* allele was 0.275, with the **1* allele frequency being 0.725. The frequency of the *CYP2B6*1/*1*, *CYP2B6*1/*6* and *CYP2B6*6/*6* genotypes were 0.55, 0.35, 0.1, respectively.

**Conclusions:** This is the first investigation to report the frequencies of *CYP2B6*/6* alleles in the Azerbaijani population. The results of this study suggest that genetic polymorphisms in the CYP2B6 gene are abundantly present among Azerbaijani individuals.

**Key words.** Cytochrome P450, CYP2B6, PCR-RFLP, Azerbaijani.

## Introduction.

Human cytochromes P450 (CYP) are a broad spectrum of 18 mammalian cytochrome families that encode 57 genes in the human genome. The P450 superfamily plays a crucial role in the metabolism of many structurally diverse molecules and is classified on the basis of amino-acid homology [1]. Two main classes functionally divide human CYPs: the first involves biosynthesis of bile acids, steroids, and fatty acids, whereas the second class includes xenobiotics metabolism primarily carried out by CYP1, CYP2 and CYP3 isoenzyme families [2]. Cytochrome gene families initially attracted interest because of their strong correlation with the metabolism of drugs, steroids, and carcinogens. Approximately 12 CYP isoenzymes of CYP1, CYP2 and CYP3 are responsible for Phase I biotransformation of drugs and various xenobiotics in the human liver. Thus, it is important to mention that the expression and function of these gene families vary dramatically both inter-and intra-individually, which defines unpredictable drug response.

CYP supergene family harbors a great number of single nucleotide variants, which are an essential source of variation. However, processes like inhibition or induction by drugs and biological and physiological conditions may limit their penetrance [3].

Cytochrome P450 2B6 is one of the less-characterized human isoforms. CYP2B6 gene has been located on chromosome 19q13.2 and is known for its participation in the metabolism of many drugs such as isophamine, tamoxifen, ketamine, propofol, imatinib, efavirenz and several environmental carcinogens [1]. Initially, the lack of knowledge regarding substrates and inhibitors held back the research of CYP2B6's phenotypic characterization and pharmacogenetic effects. Currently, 40 alleles and more than 30 amino-acid altered single-nucleotide polymorphisms are identified for the CYP2B6 gene, which determinates its highly expressed nature. Moreover, it has been reported that CYP2B6 demonstrates a possible significance in clinical drug treatment. For instance, CYP2B6 allele 516G>T is associated with greater plasma of exposure to efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor drug used in human immunodeficiency virus (HIV) treatment. CYP2B6 also plays an important role in the metabolism of nevirapine (another drug used in HIV treatment) [4,5].

Among gene polymorphisms, CYP2B6 c.516G>T is one of the most common exonic alterations. CYP2B6*6 was documented to have a relationship with different types of blood malignancy. It has been studied in different populations, but ethnicity is a crucial variable to account for interindividual variability.

Thus, this study was aimed to investigate the genotype and allelic frequencies of G15631T SNP in an Azerbaijani population, as the determination of SNP's prevalence will be useful in further pharmacogenetics research.

## Materials and methods.

### Subjects and DNA extraction

Our investigation included 100 individuals (68 females and 32 males) aged 15-81 years (mean: 36; standard deviation: 16), randomly selected from various regions of the country and recruited from the Institute of Hematology and Transfusiology, named after B. Eyvazov. The study was approved by the Ethics Committee of the Institute of Hematology and Transfusiology, named after B. Eyvazov, which complies with the Declaration of Helsinki of 1964, as revised in 2013. The protocol of written informed consent was obtained from all the patients involved in the investigation.

The investigation consisted of individuals who belong to the Azerbaijani ethnic group and have resided in Azerbaijan for three consecutive generations. The subjects with a history or evidence of hepatic or hematological abnormalities, hepatitis B or C or HIV infection, or any other acute or chronic disorders were not included.

Due to established inclusion criteria, our study involved 100 unrelated volunteers who were admitted to IHT between February 2017 and December 2019.

Two milliliters' peripheral blood samples in EDTA tubes were collected from the volunteer group. Genomic DNA was

isolated using a DNA extraction kit, QIAGEN QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Genotyping was conducted at the Institute of Genetic Resources of Azerbaijan National Academy of Sciences. DNA quality and quantity were measured by NanoDrop 2000c Spectrophotometers (Thermo Scientific).

**Genotyping of CYP2B6 Polymorphisms**

The PCR amplification for *CYP2B6* was performed by using the primer pair, forward 5'-CTGTGTCCTTGACCTGCTGC-3', and reverse 5'TCCAGGAGCAGAATAGACATGAAG-3'. The mixture consisted of 2.5 µl of 10x PCR buffer, 2.0 µl $MgCl_2$, 0.25 µl dNTPs, 0.25 FIREPOL Taq Polymerase, 0.5 µl of each primer, 16 µl deionized water and 3 µl DNA. The amplification was initiated with denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30s, 56°C for 1 minute, 72°C for 2 minutes and a final extension step at 72°C for 5 min. The product of amplification was analyzed on a 1.5% agarose gel at 110 V for 35 minutes.

The digestion of 2.5 µl of PCR products was carried with 0.5 units of restriction enzyme Bsr1 (New England BioLabs, BioLabs) for an hour at 65°C and the genotyped on a 3% agarose gel. The homozygote wild-type variant (GG) was identified by the presence of two bands on 518 bp and 60-bp fragments, whereas the homozygote mutant variant (TT) produced a band at 578-bp, and heterozygote (GT) revealed three bands at 578, 518, and 60 bp (Figure 1).

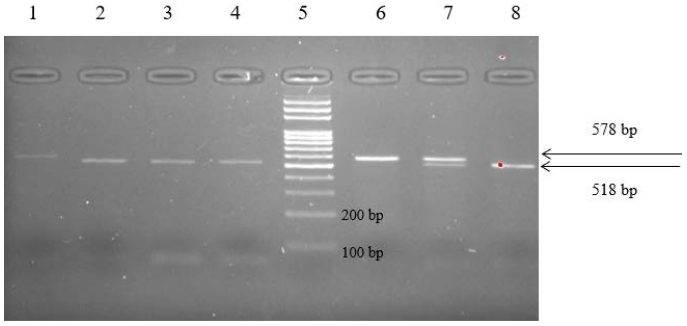


***Figure.1.*** *Gel electrophoresis for CYP2B6*6 (digestion enzyme Bsr1). Lane 5 contains a 100-bp ladder. Lanes 2 to 4 and 8 indicate a homozygous wild-type variants (GG). Lanes 1 and 6 show a homozygous mutant individual (TT). Lane 7 demonstrates heterozygous genotype (GT).*

**Sequencing**

Following genotyping, 10% of all samples from each different genotype were randomly selected for confirmation sequencing. The PCR products were purified by using a QIAquick PCR and then sent to INTERGEN Laboratory (Ankara, Turkey) for confirmation via next-generation sequencing.

**Statistical Analysis**

The chi-square test ($\chi^2$) was applied in order to compare the frequencies of polymorphic genotypes and alleles. The deviations from the Hardy-Weinberg equilibrium for allele and genotype frequencies for the different SNPs were assessed by Fisher's Exact Test.

All the statistical tests were two-sided; the level of significance was taken as $p<0.05$.

Statistical analysis was carried out using the SPSS package (ver. 22, SPSS, Chicago, IL).

## Results.

The experiment involved DNA samples of one hundred healthy Azerbaijani volunteers. Genotype and allelic frequencies of *CYP2B6*6* polymorphism were identified using the PCR-RFLP technique. The frequency of the **6* allele was 0.275, with the **1* allele frequency being 0.725. The frequency of the wildtype homozygote (*CYP2B6*1/*1*), heterozygote (*CYP2B6*1/*6*) and mutant homozygote (*CYP2B6*6/*6*) genotypes were 0.55, 0.35, 0.1, respectively. The given values conformed well with Hardy-Weinberg equilibrium predictions.

The data of comparison of the allelic and genotype frequencies between the Azerbaijani population and other reported ethnic populations are shown in the Table 1.

***Table 1.*** *Chi-square test and P-values of differences in allelic frequencies between Azerbaijani and various ethnic groups.*

| ***Population*** | ***Azerbaijani*** | |
|---|---|---|
| | ***Chi-square test ($\chi^2$)*** | ***P-value*** |
| Turkish | 71.443 | <0.01 |
| British | 46.496 | <0.01 |
| Spanish | 27.352 | <0.01 |
| Canadian | 62.069 | <0.01 |
| Japanese | 22.792 | <0.01 |
| Han Chinese | 51.637 | <0.01 |
| Southern Chinese | 53.627 | <0.01 |
| Korean | 23.466 | <0.01 |
| African | 23.378 | <0.01 |

The allelic frequency of *CYP2B6*6* in the Azerbaijani population (27.5%) was relatively similar to Caucasian populations including British (28.1%) [6] ($\chi^2$=46.496, P<0.01), Spanish (26.5%) [7] ($\chi^2$=27.352, P<0.01), Turkish (28%) [8] ($\chi^2$=71.443, P<0.01), and Canadian (25,4%) [9] ($\chi^2$=62.069, P<0.01), however, significantly higher than Asian populations, namely Japanese (14,4%), ($\chi^2$=22.792, P<0.01), Korean (15,2%) ($\chi^2$=23.466, P<0.01) [4], Han Chinese (21%) [10] ($\chi^2$=51.637, P<0.01), excluding Southern Chinese (34%) [5] ($\chi^2$=53.627, P<0.01), and notably lower than African (42%) [11] ($\chi^2$=26.378, P<0.01). All the differences were found to be statistically significant. Correspondingly, *CYP2B6*6* mutant homozygote and heterozygote genotype frequencies were relatively similar to British ($\chi^2$=3.673, P=0.016), Turkish ($\chi^2$=0.292, P=0.476), Japanese ($\chi^2$=3.399, P=0.065) and Korean ($\chi^2$=1.910, P=0.165) populations, but significantly lower than in Africans ($\chi^2$=0.302, P=0.566). Interestingly, the comparison of the prevalence of heterozygous and mutant homozygous alleles between the Azerbaijani and Spanish populations revealed a variance ($\chi^2$=0.230, P=0.647). Namely, heterozygous genotype was significantly higher whereas mutant homozygous notably lower than in Azerbaijani population. No significant difference was found between results for this investigation and Asian, Caucasian or African populations, except for British (Table 2).

***Table 2.*** *CYP2B6 SNPs frequencies (%) observed in this study and comparison with other populations.*

| ***Population*** | ***Allele frequency*** | |
|---|---|---|
| | ***N*** | ***CYP2B6*6*** |
| Turkish | 344 | 0.253 |
| British | 270 | 0.281 |
| Spanish | 200 | 0.265 |
| Canadian | 354 | 0.254 |
| Japanese | 90 | 0.144 |
| Han Chinese | 386 | 0.21 |
| Southern Chinese | 1014 | 0.345 |
| Korean | 92 | 0.152 |
| African | 82 | 0.488 |

## Discussion.

Although CYP2B6 belongs to the poorly characterized enzymes, it is already known for over 40 highly polymorphic alleles, which makes it clinically important as alterations in CYP2B6 activity affect drug pharmacokinetics, toxicity and response [4]. As the interest towards Cytochrome P450 2B6 grows, it provokes investigations that rapidly broaden the list of known substrates. Namely, anti-cancer agents like cyclophosphamide and ifosfamide or the antiretrovirals like efavirenz and nevirapine. Also, it is worth noting narcotics, including propofol and ketamine, and a list of the antidepressants like bupropion or sertraline. Along with playing a vital role in a number of drug metabolism, it is also metabolizing some endogenous molecules such as testosterone, procarcinogens and recreational drugs [12,13]. Nevertheless, there is a lack of data related to the effects of SNPs of CYP2B6 on enzymatic activity or comparisons among reported allelic variants. Thus, CYP2B6 allele prevalence data is of great interest among different ethnic populations, especially in those in which CYP2B6 plays a significant role in drug metabolism [8].

Available data across populations demonstrate a considerable ethnic variation in the prevalence of CYP2B6 variant alleles. *CYP2B6*6* allele, which is defined by two SNPs (516G>T, 785A>G), was reported in Caucasians, with relatively similar frequencies from 25 to 28%, and in Africans, namely in West Africans and Ghanaians with frequencies of 42% and 48%, respectively; however, data varies significantly among Asian populations from 14 to 34% [5]. It is important to note that, *CYP2B6*6* allele is present in the Azerbaijani population with a frequency of 0.275, which despite the small size of the cohort, corresponds with previously collected frequencies in Turkish, British, Spanish, and Canadian populations.

For comparison, *CYP2B6*6* was less common in Asians but more frequent in Africans.

The association of CYP2B6 alterations with disruptions in the biotransformation of therapeutically important drugs are gaining evidence in many ethnically diverse populations. There are several studies to show relevance for drug therapy with CYP2B6 substrates. Namely, an in vitro study of the impact of 516G>T on expression and function in the human liver showed that the given SNP influences enzyme expression levels and activity [14]. In addition, CYP2B6 is known as a strong predictor of high systemic exposure to efavirenz (EFV) in HIV-infected patients. A recent investigation of the association between plasma EFV levels and allele *CYP2B6*6* showed that 516G>T correlates with greater plasma EFV exposure during the first 24 weeks of antiretroviral therapy and an increase in central nervous system side effects, especially during the first week. In addition, the mean plasma EFV concentrations of patients with *CYP2B6 *6/*6* genotype (516TT genotype together with 785GG genotype) were significantly higher than those of patients with *6 heterozygous genotypes and non-*6 alleles [15]. Another example of antiretroviral therapy for HIV patients is nevirapine (NVP), which treatment response has associations with the CYP2B6 genotype [16,17]. Namely, 516G>T is reported to influence patient exposure to NVP [18,19]. An investigation of bupropion (an approved therapy of nicotine dependence) exposure and CYP2B6 demonstrated that although the presence of the *CYP2B6*6* allele appeared to have no significant effect on bupropion exposure, hydroxylation of both enantiomers was higher in patients carrying *CYP2B6*6/*1*. However, contradictory results have been reported on metabolic activation of the prodrug cyclophosphamide in hepatic microsomes from *CYP2B6*6* carriers; nevertheless, lower 4-hydroxy-cyclophosphamide production and poor treatment response to cyclophosphamide was observed in patients with *CYP2B6*6* allele than in *CYP2B6*1* carriers [20]. In addition, it is interesting to mention the catalytic activity of CYP2B6 variants, which causes interindividual varieties in the hepatic metabolism of certain drugs. Namely, the presence of the *CYP2B6*6* allele decreased hepatic clearance of ketamine up to 89% in liver microsomes and 55% in c-DNA-expressed in vitro proteins [12].

Another study of ketamine clearance demonstrated that the *CYP2B6*1/*1* genotype confers a 6-fold higher clearance of both enantiomers of ketamine compared to the *CYP2B6*6/*6* genotype [14].

Our findings revealed allele and genotype frequencies of highly polymorphic CYP2B6, demonstrating the necessity for further research within the Azerbaijani population in larger cohorts as these variants may significantly affect the pharmacokinetics and pharmacodynamics properties of a number of drugs.

To our knowledge, this is the first investigation to document the frequencies of *CYP2B6*/6* alleles in the Azerbaijani population. The results of this study suggest that genetic polymorphisms in the CYP2B6 gene are abundantly present among Azerbaijani individuals.

## Conclusions.

In conclusion, our findings aim to contribute to a better understanding of the ethnic differences in CYP2B6 genotypes. Also, it shows that pharmacogenetic testing of drug metabolizing CYP enzymes may lead to improvement and optimization of personalized drug therapy in Azerbaijan.

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## Цель исследования

Несмотря на то, что цитохром P450 2B6 является одной из менее изученных изоформ человека, он уже известен своим участием в метаболизме многих медикаментов и даже нескольких канцерогенов. Он был изучен в разных популяциях, однако этническая принадлежность является важной переменной для учета межиндивидуальной изменчивости. Данное исследование было направлено на изучение частот генотипа и аллелей полиморфизма цитохрома CYP2B6 516G>T в Азербайджанской популяции, поскольку определение распространенности данной точечной мутации может принести вклад в дальнейшие фармакогенетические исследования и оптимизацию персонализированной лекарственной терапии в Азербайджане.

## Материалы и методы

Идентификацию частот аллелей и генотипов *CYP2B6*6* у 100 добровольцев проводили с использованием метода полимеразной цепной реакции-полиморфизма длины рестрикционных фрагментов (ПЦР-ПДРФ). Полученные результаты были подтверждены секвенированием нового поколения.

### Результаты

Частота аллеля *6 составила 0,275, частота аллеля *1 — 0,725. Частота генотипов *CYP2B6*1/*1, CYP2B6*1/*6 и CYP2B6*6/*6* составлает 0,55, 0,35, 0,1 соответственно.

### Заключение

Это первое исследование, в котором представляются частоты аллелей *CYP2B6*/6* в Азербайджанской популяции. Результаты этого эксперимента показывают, что генетические полиморфизмы в гене CYP2B6 в изобилии присутствуют среди азербайджанцев.

**Ключевые слова:** Цитохром P450, CYP2B6, ПЦР-ПДРФ, Азербайджан