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In-Depth Pharmacogenomic Characterization of the Serbian Population

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ABSTRACT

Pharmacogenomics enables the prediction of individual drug responses based on genetic profiles, representing a critical step toward personalized medicine through routine genetic testing. Advances in high-throughput sequencing technologies have facilitated the simultaneous identification and interpretation of variants across multiple pharmacogenes. However, incorporating pharmacogenomics into clinical practice remains challenging, partly due to limited knowledge of population-specific pharmacogenetic data. This study aimed to construct the most comprehensive pharmacogenomic map of the Serbian population to date. Genomic data from 881 Serbian individuals, obtained via clinical and whole exome sequencing, were analyzed. Raw sequencing data were processed using a custom pipeline for alignment and variant calling. Pharmacogenetic star alleles and predicted phenotypes were annotated using PharmCAT and Stargazer. Frequencies of star alleles and phenotypes were calculated and compared to both European and global populations. Population differentiation was assessed through Wright's fixation index (F_ST). The analysis revealed the greatest population differentiation between Serbians and the global population. Among Serbians, key pharmacogenes with notable star allele frequencies and clinically actionable phenotypes included CYP2B6, NAT2, SLCO1B1, UGT1A1, and VKORC1, exhibiting distribution patterns significantly distinct from other European populations. The observed variations in pharmacogenetic phenotypes, which impact responses to drug classes such as statins and antidepressants, highlight the potential benefit of integrating drug-response data into genetic reports for the Serbian population. Pharmacogenomic implementation could be efficiently achieved using existing clinical and whole exome sequencing datasets.

Keywords: High-throughput sequencing, Pharmacogenomics, Bioinformatics, Personalized medicine, Population genetics

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Introduction

Pharmacogenomics (PGx), a key component of precision medicine, aims to optimize drug efficacy and safety based on the individual genetic profile of patients and has significantly advanced due to technological progress [1]. The widespread adoption and cost reduction of next-generation sequencing (NGS) have enabled a move away from uniform treatment strategies toward approaches that account for patients' unique genetic variations [2]. Currently, clinical PGx implementation primarily targets single genes or variant panels with well-established effects on drug response [3, 4]. This clinical integration relies on evidence-based prescribing guidelines issued by pharmacogenomics consortia, notably the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG), which provide scoring systems and evidence levels for gene-drug pairs. Similarly, the Pharmacogenomics Knowledge Base (PharmGKB) offers a comprehensive framework for ranking pharmacogenomic evidence.

Despite the substantial availability of data on genetic influences on drug response, PGx adoption in routine clinical practice remains limited [5]. NGS is widely applied in molecular diagnostics, generating vast amounts of genomic data that can be leveraged to identify and interpret variants and haplotypes—commonly referred to as star alleles—

that affect drug metabolism and efficacy [6]. Increasing sequencing throughput and declining costs create opportunities for rapid, cost-effective, and preemptive PGx profiling.

Population-specific genomic differences are critical for effective clinical PGx application [7, 8]. Previous studies have characterized the Serbian genome, revealing distinctive features within this population [9, 10]. Residual data from routine diagnostic sequencing can be harnessed to delineate pharmacogenomic profiles at the population level [11-13]. Detailed characterization of subpopulations supports improved risk stratification and informs the development of population-tailored genotyping strategies [7, 8, 14].

Technological progress has fueled the adoption of population-scale precision medicine, with several Western Balkan countries reporting distinct pharmacogenomic patterns [12, 15-19]. In Serbia, numerous studies have examined drug response in conditions such as pediatric acute lymphoblastic leukemia [20, 21], inflammatory bowel disease [22, 23], and rheumatoid arthritis [24, 25]. However, a comprehensive map of pharmacogenomic variation across the Serbian population is still lacking. Leveraging the substantial NGS data generated from patients tested for rare genetic disorders presents an opportunity to explore pharmacogenomic patterns at a population level [22, 26, 27].

In this study, we aimed to characterize the pharmacogenomic landscape of the Serbian population by examining over 50 pharmacogenes using large-scale NGS datasets and PGx-focused bioinformatics tools. Our objectives included determining the frequency of star alleles and associated PGx phenotypes, comparing these findings with global and European data, and identifying population-specific PGx traits. Additionally, we assessed the utility of clinical exome sequencing (CES) and whole exome sequencing (WES) for detecting PGx variants relevant to therapeutic decision-making.

Materials and Methods

Subjects and exome sequencing

This study utilized genetic data from 881 Serbian individuals. DNA was extracted from blood samples and sequenced using either clinical or whole exome panels over a two-year period (2023–2024). For this analysis, all data were anonymized, with sex retained only for G6PD genotyping. Phenotypic information was not available. The study was approved by the Ethics Committee of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade (O-EO-046/2023).

Most samples (n = 768) were analyzed with a clinical exome sequencing panel (TruSight One, Illumina, CA, USA), covering selected exons of 4,813 clinically relevant genes. The remaining 113 samples underwent whole exome sequencing (Illumina DNA Prep with Exome 2.0 Plus Enrichment, Illumina, CA, USA), providing broader coverage of coding regions and key intronic sites.

Bioinformatic analysis

NGS raw data were processed via an in-house pipeline adhering to GATK Best Practices [28]. Reads were aligned to the hg38 reference genome using bwa-mem, and resulting BAM files underwent duplicate marking and base quality score recalibration. Variant calling was performed using HaplotypeCaller to generate gVCFs, grouped by sequencing panel type (clinical or whole exome). Joint genotyping and filtering created multi-sample VCF files for downstream analysis, ensuring inclusion of all known PGx variants via the --force-output-intervals flag and genomic positions from PharmCAT [29]. Processed VCF files were used in PharmCAT for pre-processing, allele matching, and phenotype assignment, with output organized using json2tsv and custom R scripts for statistical analysis.

Additionally, individual BAM and VCF files were analyzed using Stargazer v2.0 [30], which detects star alleles, including complex structural variants, using read depth comparisons between target pharmacogenes and control genes (--target-gene and --control-gene flags). BAM-derived depth information was formatted into GATK-DepthOfCoverage (GDF) tables, combined with VCF annotations to identify PGx variants.

Both PharmCAT and Stargazer ultimately provided diplotypes (pair of haplotypes or star alleles) and associated PGx phenotypes for each pharmacogene in each sample.

Pharmacogenetic study design

Genotyping of pharmacogenes was conducted using two complementary tools: PharmCAT [19] and Stargazer [30]. PharmCAT functions as an annotation platform that interprets VCF-derived genetic data for 18 genes referenced in CPIC and DPWG guidelines (ABCG2, CACNA1S, CFTR, CYP2B6, CYP2C19, CYP2C9,

CYP3A4, CYP3A5, CYP4F2, DPYD, G6PD, IFNL3, NUDT15, RYR1, SLCO1B1, TPMT, UGT1A1, VKORC1), translating genomic variants into star alleles and assigning phenotypes based on diplotype information. The software generates reports containing genotype-guided drug prescribing recommendations.
Stargazer expands on PharmCAT's capabilities by identifying haplotypes not only in the same genes but also in 39 additional pharmacogenes (including the CYP2C cluster, ABCB1, CYP17A1, CYP19A1, CYP1A1, CYP1A2, CYP1B1, CYP26A1, CYP2A13, CYP2A6, CYP2C8, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2W1, CYP3A43, CYP3A7, CYP4A11, CYP4A22, CYP4B1, GSTM1, GSTP1, NAT1, NAT2, POR, PTGIS, SLC15A2, SLC22A2, SLCO1B3, SLCO2B1, SULT1A1, TBXAS1, UGT1A4, UGT2B7, UGT2B15, XPC).
Because the clinical exome sequencing (CES) panel does not fully cover all exons, for genes lacking complete coverage, only whole exome sequencing (WES) data were used—specifically for the CYP2C cluster, CYP2S1, GSTM1, NUDT15, and SLC15A2. Additionally, certain well-characterized pharmacogenetic variants are located in intronic regions, such as the UGT1A1 TATA box short tandem repeats; for these variants, frequencies were calculated separately for CES and WES datasets. For genes fully captured by both CES and WES, data were combined to calculate overall allele frequencies. **Table 1** summarizes which genes were represented by clinical exome, whole exome, or both sequencing platforms.

Table 1. Lists of genes for which variant frequencies were calculated using either data from clinical exome, whole exome or both sequencing panels.

Sequencing panel	**Clinical exome sequencing data**	**Whole exome sequencing data**	**Whole exome sequencing data exclusively**	**Clinical exome + whole exome sequencing data**
Number of samples	768	113	113	881
List of genes		*CYP2C cluster*		*ABCB1*
		CYP1A2		*ABCG2*
		CYP2A6		*CFTR*
		CYP2B6		*CYP1A1*
		CYP2C19		*CYP1B1*
	CYP1A2	*CYP2D6*		*CYP2A13*
	CYP2A6	*CYP2E1*		*CYP2C8*
	CYP2B6	*CYP2J2*		*CYP2C9*
	CYP2C19	*CYP2S1*	*CYP2C* cluster	*CYP2F1*
	CYP2D6	*CYP3A4*	*CYP2J2*	*CYP2W1*
	CYP2E1	*CYP3A5*	*CYP2S1*	*CYP3A7*
	CYP3A4	*CYP4A22*	*CYP3A5*	*CYP3A43*
	CYP4A22	*DPYD*	*GSTM1*	*CYP4A11*
	DPYD	*GSTM1*	*IFNL3*	*CYP4B1*
	NAT1	*IFNL3*	*NUDT15*	*CYP4F2*
	SLCO1B1	*NAT1*	*PTGIS*	*CYP19A1*
	SULT1A1	*NUDT15*	*SLC15A2*	*CYP26A1*
	TBXAS1	*PTGIS*	*VKORC1*	*G6PD*
	UGT1A1	*SLC15A2*		*GSTP1*
	UGT1A4	*SLCO1B1*		*NAT2*
		SULT1A1		*POR*
		TBXAS1		*RYR1*
		UGT1A1		*SLC22A2*
		UGT1A4		*SLCO1B3*
		VKORC1		*SLCO2B1*

		TPMT
		UGT2B7
		UGT2B15
		XPC

Population genetic comparative analysis and statistical methods

All statistical analyses were conducted using R software version 4.3.0. Quality control of genotyping data was performed with the HardyWeinberg package [31]. For each star allele, Hardy-Weinberg equilibrium (HWE) was assessed by comparing the observed number of allele carriers to all other alleles.

The population-level pharmacogenomics study involved comparing Serbian star allele frequencies with those from global, European, and Croatian populations. Frequency data for single-variant-defined star alleles in global and European populations were obtained from CPIC and gnomAD databases [32]. Haplotypes defined by multiple variants, however, were not directly available from these databases; their frequencies were instead calculated using the LDpair Tool [33], which estimates frequencies of variants in linkage disequilibrium based on 1000 Genomes Project data. For the Croatian population, PGx variant frequencies were extracted from a previously published population study [17] for comparison with Serbian data.

Frequency comparisons were performed using Chi-squared tests when all expected cell counts in 2 × 2 tables were ≥5; otherwise, Fisher's Exact Test was applied. Population similarity was quantified using Wright's fixation index (Fst), calculated via the bigsnpr package [34]. Predicted pharmacogenetic phenotypes provided by Stargazer and PharmCAT for actionable star alleles were compared with European subpopulations from the 1000 Genomes Project (Central European, Finnish, British, Iberian, and Tuscan populations) (Sherman, Claw, and Lee, 2024) using Chi-squared or Fisher's Exact tests. To control for multiple testing and reduce false positives, Bonferroni correction was applied by dividing 0.05 by the number of pharmacogenes included in population comparisons.

Results and Discussion

Quality control and hardy-weinberg equilibrium

Mean target coverage for clinical exome samples was 90×, while whole exome samples averaged 73×, with 92% and 86% of positions, respectively, achieving coverage ≥30×. HWE was used as a measure of genotyping quality. The significance threshold for each dataset was adjusted according to the number of pharmacogenes with at least two alleles: $p < 0.001$ (0.05/47) for CES, $p < 0.0009$ (0.05/54) for WES, and $p < 0.002$ (0.05/30) for combined panels. Pharmacogenes with variants deviating from HWE were excluded from further analysis. In cases where a variant violated HWE in CES but not in WES, WES data were retained. Genes with only reference alleles detected (CACNA1S, CYP17A1, CYP2R1) were also excluded from subsequent comparisons.

Pharmacogenomics of the serbian population

Pharmacogenetic annotation was performed using PharmCAT [19] and Stargazer [30]. Discrepancies in haplotype calls between the two tools were observed **(Table 2)** and further verified by inspecting BAM files. For downstream population-level analyses, annotations from the tool providing accurate results based on BAM data were retained **(Table 2)**. When critical intronic variants could only be detected with WES, only WES data were included in analyses **(Table 1).** Following removal of misannotated pharmacogenes, Stargazer identified 170 haplotypes across 47 pharmacogenes, whereas PharmCAT detected 62 star alleles within 13 pharmacogenes.

Table 2. Pharmacogenes annotated with PharmCAT or Stargazer based on tool performance after inspecting BAM files. Discordance in haplotype (star allele) calling between PharmCAT and Stargazer is represented in parenthesis (CES | WES).

Optimal annotation – both PharmCAT and Stargazer	Higher accuracy - PharmCAT	Higher accuracy - Stargazer
ABCG2		
CACNA1S		
CYP2C9	*CFTR* (0.3% | 0.0%)	*CYP2B6* (11.1% | 23.0%)

CYP3A5	*CYP2C19* (27.5% | 5.3%)	*CYP3A4** (NA | 1.8%)
G6PD	*CYP4F2** (NA | 67.3%)	*DPYD* (14.9% | 3.1%)
IFNL3	*RYR1* (100% | 100%)	*SLCO1B1* (8.9% | 0.0%)
NUDT15	*UGT1A1* (0.0% | 11.5%)	
TPMT		
VKORC1		

*Stargazer identified only the *3 and *4 alleles of CYP4F2 in both CES and WES datasets, whereas PharmCAT showed poor performance on CES, with 232 out of 1,536 values reported as NA; consequently, only WES data were used for cross-tool comparisons. Likewise, for CYP3A4, both Stargazer and PharmCAT performed suboptimally on CES data, so analyses relied solely on WES results.

According to Stargazer, CYP2D6 exhibited the highest number of alternative haplotypes (N = 12), although PharmCAT did not provide haplotype information for this gene. In contrast, PharmCAT identified RYR1 as the most polymorphic pharmacogene, reporting 18 alternative haplotypes. Across the 52 pharmacogenes analyzed using either Stargazer or PharmCAT, the greatest variability—defined as the proportion of non-reference haplotypes—was observed in CYP3A5 (93%) and CYP2C19 (92%). The pharmacogenes with the lowest variability included CYP26A1 (0.1%), CFTR (0.5%), G6PD (1.0%), PTGIS (1.3%), NUDT15 (1.3%), TBXAS1 (3.1%), and TPMT (3.3%). Considering both pharmacogenomic actionability and allele frequencies in the Serbian population, the most clinically relevant variants were CYP2C9*2, G6PD Gond, NAT2*7, SLCO1B*15, SLCO1B1*14, SLCO1B1*15, UGT1A1*6, UGT1A1*28, UGT1A1*36, and VKORC1 rs9923231 **(Table 4).**
Following review of PGx annotations from both PharmCAT and Stargazer, the sequencing panels used in this study were evaluated for their suitability in pharmacogenomic testing **(Table 3).** Of the 20 pharmacogenes considered actionable for drug response and therapy optimization according to CPIC guidelines, CES provided adequate coverage for only 7 genes. For the remaining 13 pharmacogenes, which harbor variants critical for drug response, CES coverage was insufficient, making WES necessary to capture the relevant genetic information **(Table 3).**

Table 3. Pharmacogenes annotated by PharmCAT or Stargazer that have CPIC published guidelines and can be analysed using clinical exome or whole exome sequencing panels.

Clinical and whole exome sequencing	Whole exome sequencing
	CACNA1S
	CFTR
	CYP2B6
ABCG2	*CYP2D6*
CYP2C8	*CYP2C19*
CYP2C9	*CYP3A4*
G6PD	*CYP3A5*
RYR1	*CYP4F2*
SLCO1B1	*DPYD*
TPMT	*IFNL3*
	NUDT15
	UGT1A1
	VKORC1

Comparison of the serbian population with other populations
In total, 209 star alleles were identified in the Serbian cohort, and their frequencies were compared with those reported in global and European populations. Data for general and European populations were obtained from gnomAD and CPIC, while Croatian PGx haplotype frequencies were taken from a previously published study [17]. To account for multiple testing, Bonferroni correction was applied by dividing 0.05 by the 52 analyzed pharmacogenes, with significance set at $p < 0.0009$. Differences in haplotype frequencies were observed for 35 of the 52 pharmacogenes.

When compared with the global population, 60 star alleles across 31 pharmacogenes exhibited significant frequency differences in the Serbian population. SLCO1B1 and NAT1 showed the greatest number of divergent haplotypes. Alleles with higher prevalence in Serbia included SLCO1B1*5 ($p < 2.2 \times 10^{-16}$), SLCO1B1*14 ($p = 5.2 \times 10^{-6}$), SLCO1B1*19 ($p = 1.6 \times 10^{-5}$), NAT1*15 ($p = 1.1 \times 10^{-6}$), NAT1*19 ($p = 1.8 \times 10^{-5}$), and NAT1*22 ($p = 1.3 \times 10^{-5}$). Conversely, some alleles were more frequent in the worldwide population, including SLCO1B1*15 ($p = 3.4 \times 10^{-7}$), SLCO1B1*37 ($p < 2.2 \times 10^{-16}$), and NAT1*17 ($p = 7.6 \times 10^{-4}$).

Compared with European populations, 48 star alleles across 29 pharmacogenes showed statistically significant differences. Consistent with global comparisons, NAT1 exhibited the most alleles with differing frequencies: higher in Serbia (NAT1*15 $p = 4.2 \times 10^{-6}$, NAT1*19 $p = 8.4 \times 10^{-6}$, NAT1*22 $p = 5.9 \times 10^{-5}$) and higher in Europe (NAT1*17 $p = 6.31 \times 10^{-5}$).

Haplotype frequencies were also evaluated against the Croatian population for 14 pharmacogenes. Due to differences in genotyping panels, only shared variants were compared. Frequency differences were observed for 4 pharmacogenes: CYP2D6, CYP4F2, SLCO1B1, and UGT1A1. The most notable disparity was observed in SLCO1B1, with significantly lower allele frequencies in Serbia (SLCO1B1*5 $p < 2.2 \times 10^{-16}$, SLCO1B1*20 $p = 0.006$, SLCO1B1*37 $p < 2.2 \times 10^{-16}$).

Star alleles classified as Level 1 or 2 by PharmGKB, or as level A or B by CPIC, which exhibited frequency differences between Serbia and other populations, are summarized in **Table 4.** Those with frequencies >1% in both the Serbian population and in European or worldwide populations are illustrated in **Figure 1**.

Table 4. Star alleles with significantly different frequencies in Serbian compared to worldwide or European populations. Pharmacogenes with the clinical annotation level of evidence 1 and 2 (PharmGKB) or level A and B (CPIC) were included.

Star allele	Frequency in the Serbian population (%)	Frequency in the worldwide population (%)	p value	Frequency in the european population (%)	p value
CYP2A6 *35	16.4	3.9	$<2.2*10^{-16}$	4.2	$<2.2*10^{-16}$
CYP2C9 *2	13.2	9.2	$1.3*10^{-8}$	12.8	0.7
CYP2C19 *1	53.0	93.7	$<2.2*10^{-16}$	93.1	$<2.2*10^{-16}$
CYP2D6 *35	13.3	3.9	$4.01*10^{-12}$	5.5	$5.7*10^{-7}$
G6PD A	0.1	1.7	$1.5*10^{-7}$	0.04	0.5
G6PD Gond	0.4	0.04	$5.1*10^{-6}$	0.01	$1*10^{-11}$
NAT2 *7	3.0	3.4	0.5	1.8	$4.5*10^{-5}$
NAT2 *12	0.3	59.0	$<2.2*10^{-16}$	57.0	$<2.2*10^{-16}$
NAT2 *13	0.1	33.0	$<2.2*10^{-16}$	32.0	$<2.2*10^{-16}$
SLCO1B1 *5	4.2	1.5	$<2.2*10^{-16}$	2.3	$3.9*10^{-7}$
SLCO1B1 *14	15.0	11.3	$5.2*10^{-6}$	15.3	0.8
SLCO1B1 *15	14.1	10.2	$3.4*10^{-7}$	13.0	0.2
SLCO1B1 *37	11.4	20.3	$<2.2*10^{-16}$	6.2	$<2.2*10^{-16}$
UGT1A1 *6	0.4	5.5	$<2.2*10^{-16}$	0.8	0.3
UGT1A1 *28	2.7	32.4	$<2.2*10^{-16}$	31.6	$<2.2*10^{-16}$
UGT1A1 *36	4.1	3.4	0.7	0.0	$1.1*10^{-8}$
VKORC1 rs9923231	49.6	31.9	$1.9*10^{-8}$	37.8	$4*10^{-4}$

Differences in allele frequencies were assessed using either the 2 × 2 Chi-squared test or Fisher's Exact test. To account for multiple comparisons, Bonferroni correction was applied, and results were considered statistically significant when $p < 0.0009$, both for comparisons between tools and between populations.

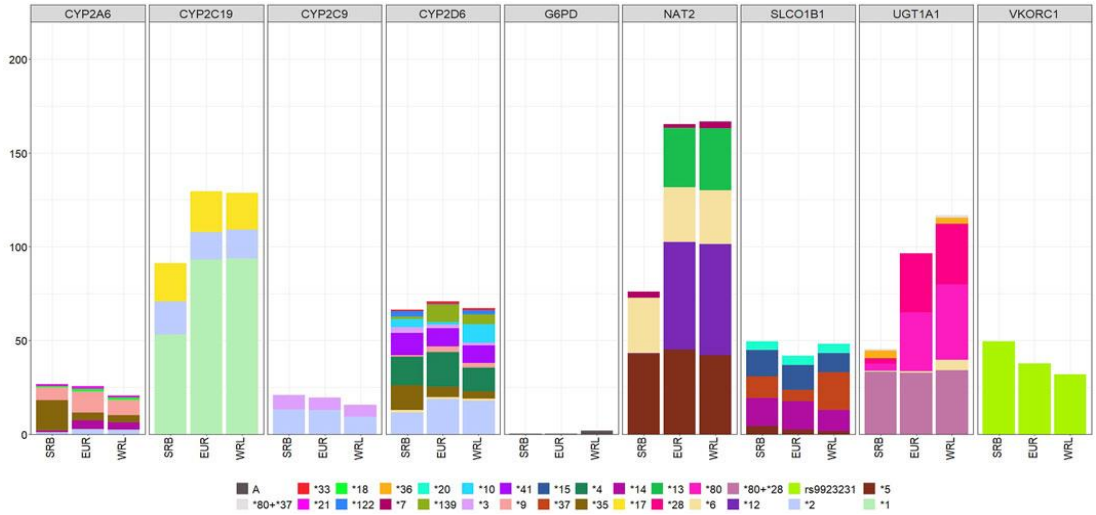

Figure 1. Level A and B pharmacogenomic variants detected in the Serbian (SRB), European (EUR), and worldwide (WRL) populations.

Population similarity among Serbian, European, and worldwide cohorts was further evaluated using Wright's fixation index (Fst), which ranges from 0 to 1, with higher values reflecting greater population differentiation. Overall, differentiation across the analyzed pharmacogenes was modest **(Figure 2)**, with the highest Fst observed between the Serbian and worldwide populations for CYP2A6 (Fst = 0.31). As anticipated, the Serbian population showed closer genetic similarity to Europeans than to the global population (mean Fst: Serbian vs. worldwide = 0.052; Serbian vs. European = 0.047). The lowest differentiation was observed between the Serbian and Croatian populations (p = 0.007).

Figure 2. Heatmap depicting Wright's fixation index (Fst), where larger values indicate greater genetic differentiation between populations. NA denotes pharmacogenes for which data from the Croatian population were unavailable. SRB-ALL shows Fst values comparing the Serbian population with the worldwide dataset,

SRB-EUR compares Serbia to European populations, and SRB-CRO compares Serbia with Croatia. Star allele frequencies for global and European populations were sourced from CPIC and gnomAD databases, whereas Croatian data were obtained from Matišić *et al*., 2023.[17]

Pharmacogenetic phenotypes in the serbian cohort

Predicted pharmacogenetic phenotypes linked to CPIC level A/B or PharmGKB level 1/2 drug categories were available for 16 of the 52 analyzed pharmacogenes **(Table 5)**. In line with European population trends, the majority of the Serbian cohort (85.8%) were classified as CYP3A5 poor metabolizers, while the remaining individuals were intermediate metabolizers **(Table 5)**. Notably, carriers of the *3/*3 diplotype, despite being categorized as poor metabolizers, are recommended to start treatment at standard doses, whereas *1/*1 and *1/*3 carriers (normal or intermediate metabolizers) require higher initial doses. NAT2 exhibited the greatest proportion of reduced-function alleles, with 94.6% of individuals carrying deleterious variants. In contrast, CYP1A2 displayed the highest frequency of predicted ultrarapid metabolizers, accounting for 84.1% of the cohort.

Table 5. Predicted phenotype frequencies of the Serbian population.

Gene	Phenotype	Frequency	Associated drug categories according to CPIC (A/B level) and PharmGKB (1/2 level)
ABCG2	Normal function	78.4	Statins
	Decreased function	20.9	
	Poor function	0.7	
CFTR	Ivacaftor non-responsive in CF patients	98.8	CFTR modulators
	Ivacaftor responsive in CF patients	1.2	
CYP1A2	Normal metabolizers	15.9	NA
	Ultrarapid metabolizers	84.1	
CYP2A6	Normal metabolizers	92.0	NA
	Intermediate metabolizers	6.2	
CYP2A13	Normal metabolizers	92.1	NA
	Intermediate metabolizers	3.5	
CYP2B6	Normal metabolizers	85.0	Antiretrovirals SSRI antidepressants opioids
	Intermediate metabolizers	5.3	
	Ultrarapid metabolizers	4.4	
CYP2C9	Normal metabolizers	60.8	NSAIDs statins Anticoagulants Anticonvulsants
	Intermediate metabolizers	36.2	
	Poor metabolizers	3.0	
CYP2C19	Normal metabolizers	41.6	Antacids SSRI and SNRI antidepressants antiplatelet medications tricyclic antidepressants antifungals
	Intermediate metabolizers	26.5	
	Rapid metabolizers	23.0	
	Ultrarapid metabolizers	3.5	
	Poor metabolizers	5.3	
CYP2D6	Normal metabolizers	54.0	norepinephrine reuptake inhibitors SSRI and SNRI antidepressants antineoplastics opioids antiemetics tricyclic antidepressants antihypertensives
	Intermediate metabolizers	36.3	
	Poor metabolizers	1.8	
CYP2F1	Normal metabolizers	41.8	NA
	Intermediate metabolizers	23.8	
	Poor metabolizers	2.5	
CYP3A5	Intermediate metabolizers	14.2	immunosuppresants

	Poor metabolizers	85.8	
CYP3A43	Normal metabolizers	72.6	NA
	Intermediate metabolizers	8.0	
CYP4A11	Normal metabolizers	98.8	NA
	Intermediate metabolizers	1.0	
CYP19A1	Normal metabolizers	99.3	NA
	Intermediate metabolizers	0.5	
DPYD	Normal metabolizers	95.6	antineoplastics and cytotoxics
	Intermediate metabolizers	4.4	
G6PD	Normal function	98.5	antibiotics antimalarials antidotes antigout agents
	Deficient	0.1	
	Variable	0.6	
IFNL3	Normal function	49.6	immunomodulators
	Decreased function	40.7	
	No function	9.7	
NAT2	Normal function	5.4	antituberculosis agents
	Decreased function	94.6	
NUDT15	Normal metabolizers	97.3	immunosuppressants antimetabolites
	Intermediate metabolizers	2.7	
POR	Normal function	51.9	NA
	Decreased function	48.0	
	Unknown function	0.1	
PTGIS	Normal function	98.2	NA
	Increased function	1.8	
SLCO1B1	Normal function	61.5	statins
	Increased function	3.6	
	Decreased function	27.1	
	Poor function	6.5	
SLCO1B3	Normal function	3.6	NA
	Decreased function	24.5	
	Poor function	71.9	
SULT1A1	Normal function	48.0	NA
	Decreased function	52.0	
TBXAS1	Normal function	94.7	NA
	Decreased function	2.7	
TPMT	Normal metabolizers	93.6	immunosupressants antimetabolites
	Intermediate metabolizers	4.9	
	Poor metabolizers	0.2	
	Possible intermediate metabolizer	0.1	
UGT1A1	Normal metabolizers	34.5	antiretrovirals
	Intermediate metabolizers	46.9	
	Ultrarapid metabolizers	8.8	

UGT1A4	Normal function	76.1	NA
	Decreased function	15.9	
VKORC1	Normal function	26.5	anticoagulants

NA, pharmacogenes for which level of evidence linking diplotype to phenotype are level 3 and 4 (PharmGKB) or level C and D (CPIC).

Predicted phenotype frequencies in the Serbian population were compared with European subpopulations from the 1000 Genomes Project (Central European–CEU, Finnish–FIN, Great Britain–GBR, Tuscans–TSI, and Iberian Spanish–IBS) [35] for 12 pharmacogenes (ABCG2, CYP2B6, CYP2C19, CYP2C9, CYP2D6, CYP3A5, DPYD, IFNL3, NUDT15, SLCO1B1, TPMT, and UGT1A1) and with the Croatian population for 10 pharmacogenes (CYP2B6, CYP2C19, CYP2C9, CYP2D6, CYP3A5, DPYD, NUDT15, SLCO1B1, TPMT, and UGT1A1). To correct for multiple testing, Bonferroni adjustment was applied by dividing 0.05 by the number of analyzed genes (12 for European comparisons, 10 for Croatian), setting significance thresholds at $p < 0.004$ and $p < 0.005$, respectively.

Significant differences in phenotype distributions between Serbia and European subpopulations were observed for ABCG2, CYP2B6, and SLCO1B1 **(Figure 3)**. The Serbian cohort showed a higher proportion of decreased-function alleles for ABCG2 compared to CEU ($p = 9.1 \times 10^{-7}$) and TSI ($p = 3.2 \times 10^{-7}$). In contrast, intermediate metabolizer phenotypes in CYP2B6 were more prevalent across all European subpopulations than in Serbia (CEU $p = 9.9 \times 10^{-11}$, FIN $p = 1.6 \times 10^{-6}$, GBR $p = 1.9 \times 10^{-7}$, IBS $p = 6.5 \times 10^{-6}$, TSI $p = 8.1 \times 10^{-10}$). For SLCO1B1, increased enzymatic activity was more frequent in all European groups except FIN when compared to the Serbian population (CEU $p = 4.6 \times 10^{-13}$, GBR $p = 4.0 \times 10^{-10}$, IBS $p = 6.2 \times 10^{-14}$, TSI $p = 1.4 \times 10^{-11}$).

Comparisons with the Croatian population revealed that CYP2D6 poor metabolizers were more common in Croatia ($p = 0.003$), whereas for SLCO1B1, the Serbian population exhibited higher frequencies of both poor ($p = 0.0008$) and rapid metabolizers ($p = 8.9 \times 10^{-5}$). Phenotype distributions for the remaining analyzed pharmacogenes were largely consistent with both European and Croatian populations.

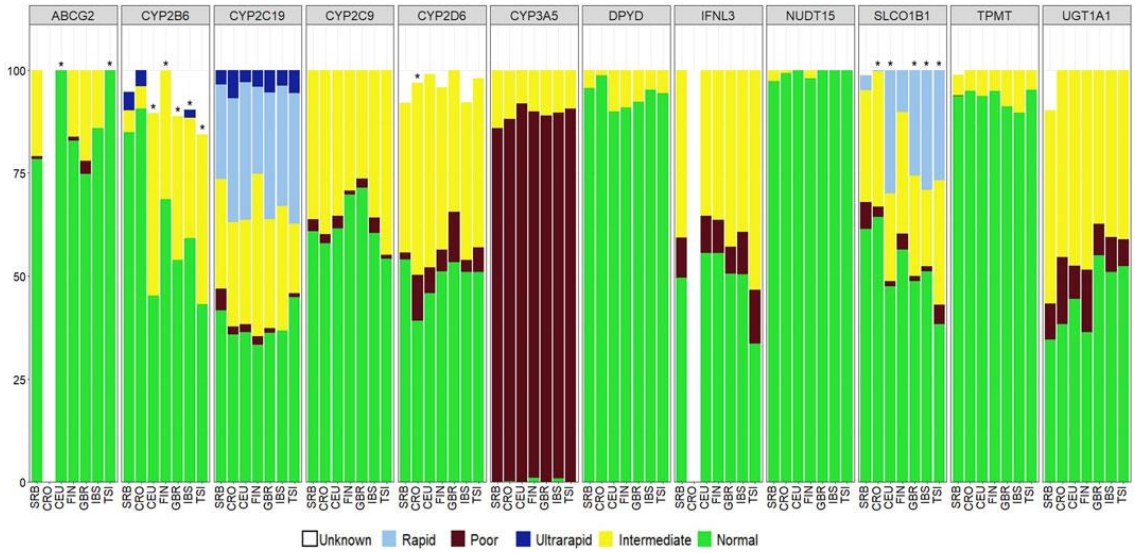

Figure 3. Pharmacogenetic phenotypes in the Serbian (SRB), Croatian (CRO), Central European (CEU), Finnish (FIN), Great Britain (GBR), Iberian Spanish (IBS), and Tuscan (TSI) populations. Statistical significance was assessed using Chi-squared and Fisher's Exact tests, with Bonferroni-adjusted thresholds set at $p < 0.004$ for SRB–EUR comparisons and $p < 0.005$ for SRB–CRO comparisons.

The rapid evolution of next-generation sequencing (NGS) has transformed both genomic research and clinical diagnostics, enabling more precise approaches to therapy optimization. Global efforts to incorporate NGS into personalized medicine have led to continuous updates of pharmacogenomic guidelines, including those from CPIC and DPWG. In this context, our study represents the first extensive evaluation of star allele frequencies across more than 50 pharmacogenes in a large cohort of Serbian individuals, providing crucial insights into potentially population-specific pharmacogenetic markers suitable for preemptive and predictive PGx testing in Serbia.

A major aim of this work was to assess the utility of clinical exome sequencing (CES) and whole exome sequencing (WES) for pharmacogenomics profiling. For 29 pharmacogenes, both CES and WES enabled successful variant annotation, demonstrating that either platform can be applied for pharmacogenomic assessment. Among these, seven pharmacogenes included in CPIC dosing guidelines (ABCG2, SLCO1B1, CYP2C8, CYP2C9, G6PD, RYR1, TPMT) could be reliably assessed via CES, highlighting its cost-effective potential for broad pharmacogenetic testing. Nonetheless, for comprehensive PGx profiling, WES provided superior coverage, capturing all relevant pharmacogenes and critical variants (CYP2C1917, CYP3A53, NUDT15, UGT1A1 TATA box, VKORC1). For detecting complex structural rearrangements, particularly in CYP2D6, whole genome sequencing (WGS) remains necessary, as our study only identified single nucleotide variants and small indels despite the large cohort. Overall, CES and WES are practical for clinical pharmacogenomics for many actionable pharmacogenes, particularly those included in drug dosing guidelines.

In addition, we evaluated the performance of two widely used pharmacogenomic annotation tools, PharmCAT and Stargazer. Both tools were applied to 18 pharmacogenes, revealing discordant haplotype calls in nearly half of the genes analyzed. Discrepancies arose primarily from outdated or incomplete variant databases in Stargazer (e.g., RYR1 and CFTR 711+3A>G) or PharmCAT (e.g., DPYD) and from ambiguous phasing of complex haplotypes in unphased datasets of unrelated individuals. In these cases, diplotypes were selected based on the most probable haplotypes inferred from European population frequencies. These findings underscore the benefit of using multiple annotation tools supplemented by careful manual review and rigorous quality control in PGx studies.

The distribution of pharmacogenetic variants in the Serbian population was then compared with other populations. Pharmacogenes with actionable variants of notable frequency and included in CPIC guidelines—CYP2C9, NAT2, SLCO1B1, UGT1A1, and VKORC1—emerged as particularly relevant for inclusion in clinical genetic reports, given their influence on response to anticoagulants, antituberculosis drugs, statins, immunosuppressants, and anticancer agents. Star allele frequencies differed significantly in 31, 29, and 4 pharmacogenes when compared to worldwide, European, and Croatian populations, respectively. Most differences were observed in genes with lower levels of PGx evidence. However, 12 pharmacogenes with Level A or B evidence per CPIC guidelines (CFTR, CYP2C9, CYP2C19, CYP2D6, CYP4F2, G6PD, NAT2, RYR1, SLCO1B1, TPMT, UGT1A1, VKORC1) showed substantial differences between the Serbian and worldwide or European populations.

Notably, the Serbian population exhibited higher frequencies of certain Level A/B star alleles (CYP2D6*35, RYR1 c.4178A>G, RYR1 c.10042C>T) and lower frequencies of others (CYP2C19*1, G6PD A, NAT2*12, NAT2*13, RYR1 c.10747G>C, SLCO1B1*37) compared with the global dataset. Similar trends were observed in comparison with European populations, except that SLCO1B1*37 was also more frequent in Serbia, while CYP2C1*91, NAT2*12, NAT2*13, RYR1 c.10747G>C remained less frequent. These variants contribute to the normal or uncertain function of their respective proteins. Specifically, star alleles associated with higher frequencies in Serbia relative to other Europeans include NAT27, SLCO1B15, UGT1A1*36, and VKORC1 -1639A, whereas UGT1A1*28 showed reduced prevalence in the Serbian population.

One variant that displayed a notably higher frequency in the Serbian population compared to both worldwide and European populations was CFTR 711+3A>G. Given that the genomic data in this study were derived from individuals who underwent exome sequencing for diagnostic purposes, it is possible that some participants had a diagnosis of cystic fibrosis, which could account for the elevated frequency of this pathogenic variant in our cohort.

Additionally, our analysis revealed a significantly higher frequency of VKORC1 -1693A in the Serbian population relative to global and European datasets, in agreement with prior reports [36]. In contrast, previous observations of an increased incidence of CYP2C9*3 in Serbia [37] were not replicated in our study, with the allele frequency instead aligning with European averages. Regarding thiopurine drug response, earlier studies in Serbia examined pediatric patients with acute lymphoblastic leukemia and adult patients with inflammatory bowel disease, reporting frequencies of PGx variants in TPMT [20, 22], TYMS, SLC19A1, DHFR, ITPA, ABCC4, and ABCB1 [38]. Building on previously reported Serbian TPMT variants (*2, *3A, *3C), our study identified three additional star alleles: **TPMT9, 20, and 43.

The SLCO1B1*5 variant had been previously described in the Serbian population [21]. Earlier studies suggested a higher frequency of this allele than observed in our results. In our dataset, SLCO1B1*5 appeared both as a single variant and as part of the SLCO1B1*15 haplotype; when combining the frequencies of the individual variant and the haplotype, the overall frequency aligns with prior findings. Previous Serbian studies relied on smaller sample sizes and PCR-based genotyping, followed by Sanger sequencing or genotyping arrays [20, 21, 36-38]. Although

most studies included patients with different clinical diagnoses, variants in pharmacogenes are generally not disease-causing, suggesting that these findings reflect the true pharmacogenetic landscape of the Serbian population. Collectively, these comparisons highlight the importance of PGx testing prior to initiating treatment across diverse clinical contexts.

In the pharmacogenes CACNA1S, CYP17A1, and CYP2R1, only reference alleles were detected, consistent with expectations, as alternative alleles in these positions are rare worldwide.

Population differentiation analysis using Wright's fixation index (Fst) indicated that the Serbian population showed the greatest divergence from the global population, particularly for CYP2A6 (Fst = 0.31). As anticipated, the Serbian population demonstrated the highest similarity with the Croatian population. Due to data availability and geographical proximity within the Western Balkans, Croatia was included for a more detailed comparison. While Slovenian population data are also available [12], differing statistical approaches precluded direct comparison. Overall, frequencies of most analyzed star alleles were concordant between the Serbian and Slovenian populations, consistent with high genetic similarity indicated by Fst values.

The largest differences between Serbia and Croatia were observed for SLCO1B15, SLCO1B137, and CYP4F2*3, which were more frequent in Croatia. This discrepancy may be attributable to methodological differences: our study employed CES and WES covering broader genomic regions, whereas the Croatian study utilized a targeted panel capturing only the most clinically relevant star alleles, potentially missing alternative alleles. Additionally, the higher frequency of UGT1A1*28 reported in the Croatian population was similar to earlier Serbian findings [39]. In our study, UGT1A1*28 was detected both as an individual variant and as part of the haplotype **UGT1A180+28; combining these frequencies yields values consistent with previously reported data in both Serbian and Croatian populations.

A major component of our study involved examining pharmacogenetic phenotypes linked to various drug categories in the Serbian population. While many of these phenotypes influence protein function, evidence remains limited regarding how structural changes in proteins directly impact drug metabolism. Consequently, comparisons between the Serbian population and other European subpopulations were restricted to pharmacogenes included in established guidelines, such as those published by CPIC. We observed that a substantial proportion of the Serbian cohort (94.5%) carries NAT2 variants (*5, *6, *7) associated with reduced enzymatic activity, which may increase the risk of drug-induced liver injury in patients treated with isoniazid [40]. Notable differences were also detected in alleles affecting statin therapy response, particularly in ABCG2 and SLCO1B1. Intermediate statin metabolizers based on ABCG2 were more prevalent in the Serbian population compared to Central European and Tuscan subpopulations, whereas SLCO1B1 rapid metabolizers were less frequent. Statin hepatic uptake is primarily mediated by SLCO1B1 transporters, while ABCG2-encoded efflux transporters modulate absorption and disposition [41]. Although rapid metabolizers of SLCO1B1 were less common in Serbia, testing remains relevant, as intermediate and poor metabolizers—who constituted over 20% of our cohort—may still exhibit altered drug responses. Additionally, intermediate metabolizers of CYP2B6, characterized by diminished enzymatic function, were more frequent in the Serbian population than in all other analyzed subpopulations. This is clinically significant, as CYP2B6 influences metabolism of several antidepressants, including anxiolytics [42]. These findings suggest that pre-treatment genotyping for NAT2, ABCG2, SLCO1B1, and CYP2B6 could benefit Serbian patients receiving antituberculosis therapy, statins, or antidepressants.

A key limitation of this study was the smaller number of samples sequenced using clinical exome sequencing (CES) relative to whole exome sequencing (WES). WES was generally more effective for pharmacogenomic analyses because it includes many critical variants located in splicing regions and exons not covered by CES. Although WES captured a wide range of variants, whole genome sequencing (WGS) would provide even more comprehensive data, particularly for complex loci. For example, while we detected multiple CYP2D6 star alleles, copy number variants could not be identified. Moreover, the annotation tools employed do not assess highly polymorphic HLA genes, which are relevant for response to antiepileptic and antiretroviral drugs. Another limitation was the de-identified nature of the data, which prevented correlation with clinical diagnoses or therapy responses.

Our population comparisons relied on multiple databases, including CPIC, 1000 Genomes, gnomAD, and previously published studies. Ideally, allele frequencies would be compared using a single, harmonized dataset with matched sample sizes; however, no such resource currently exists. While frequency estimates across databases are generally consistent, differences in source populations could influence results. Biases in global datasets also pose challenges for interpreting Wright's fixation index (Fst). Both the 1000 Genomes Project and

gnomAD are enriched for European ancestry, leaving many populations underrepresented. Variability in sample sizes among populations may artificially inflate or reduce Fst values, and grouping genetically distinct subpopulations into broad categories can obscure intra-population heterogeneity, potentially masking true differentiation. These factors must be carefully considered when interpreting population-level differences, especially against global datasets.

Conclusion

In summary, this study provides a comprehensive overview of the pharmacogenomic landscape in the Serbian population, representing the largest cohort analyzed to date in Serbia for PGx purposes. Using multiple databases (CPIC, 1000 Genomes, gnomAD, and published studies), we compared allele frequencies with other populations and highlighted clinically relevant pharmacogenetic phenotypes pertinent to drugs commonly used in Serbia. Our findings reveal interethnic variability in key pharmacogenes, including NAT2, SLCO1B1, UGT1A1, and VKORC1, and offer insights into the utility of different sequencing technologies and annotation tools in PGx analysis. We demonstrated that results are often influenced by the choice of methodology and tools; therefore, careful selection of pharmacogenes, star alleles, sequencing platforms, and annotation tools is critical for accurate PGx implementation. This work supports the integration of population-specific pharmacogenomic data into personalized genetic reports tailored for the Serbian population.

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Ethics Statement: The studies involving humans were approved by the Ethics Committee of the Institute of Molecular Genetics and Genetic Engineering University of Belgrade. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

References

1. Ng D, Hong CS, Singh LN, Johnston JJ, Mullikin JC, Biesecker LG. Assessing the capability of massively parallel sequencing for opportunistic pharmacogenetic screening. Genet Med. 2017;19(3):357–61. doi:10.1038/gim.2016.105
2. Morash M, Mitchell H, Beltran H, Elemento O, Pathak J. The role of next-generation sequencing in precision medicine: a review of outcomes in oncology. J Pers Med. 2018;8(3):30. doi:10.3390/jpm8030030
3. Tafazoli A, Guchelaar H-J, Miltyk W, Kretowski AJ, Swen JJ. Applying next-generation sequencing platforms for pharmacogenomic testing in clinical practice. Front Pharmacol. 2021;12:693453. doi:10.3389/fphar.2021.693453
4. Russell LE, Zhou Y, Almousa AA, Sodhi JK, Nwabufo CK, Lauschke VM. Pharmacogenomics in the era of next generation sequencing – from byte to bedside. Drug Metab Rev. 2021;53(2):253–78. doi:10.1080/03602532.2021.1909613
5. Kabbani D, Akika R, Ahmed W, Daly AK, Cascorbi I, Zgheib NK. Pharmacogenomics in practice: a review and implementation guide. Front Pharmacol. 2023;14:1189976. doi:10.3389/fphar.2023.1189976
6. Ji Y, Yue S, McMillin GA, Lyon E. Clinical pharmacogenomics testing in the era of next generation sequencing: challenges and opportunities for precision medicine. Expert Rev Mol Diagn. 2018;18(5):411–21. doi:10.1080/14737159.2018.1461561
7. Karamperis K, Katz S, Melograna F, Ganau FP, Van Steen K, Patrinos GP, et al. Genetic ancestry in population pharmacogenomics unravels distinct geographical patterns related to drug toxicity. iScience. 2024;27(10):110916. doi:10.1016/j.isci.2024.110916

8. Zhou Y, Lauschke VM. Population pharmacogenomics: an update on ethnogeographic differences and opportunities for precision public health. Hum Genet. 2022;141(6):1113–36. doi:10.1007/s00439-021-02385-x
9. Drljaca T, Zukic B, Kovacevic V, Gemovic B, Klaassen-Ljubicic K, Perovic V, et al. The first insight into the genetic structure of the population of modern Serbia. Sci Rep. 2021;11(1):13995. doi:10.1038/s41598-021-93129-4
10. Paschou P, Drineas P, Yannaki E, Razou A, Kanaki K, Tsetsos F, et al. Maritime route of colonization of Europe. Proc Natl Acad Sci. 2014;111(25):9211–6. doi:10.1073/pnas.1320811111
11. Nunez-Torres R, Pita G, Peña-Chilet M, López-López D, Zamora J, Roldán G, et al. A comprehensive analysis of 21 actionable pharmacogenes in the Spanish population: from genetic characterisation to clinical impact. Pharmaceutics. 2023;15(4):1286. doi:10.3390/pharmaceutics15041286
12. Hočevar K, Maver A, Peterlin B. Actionable pharmacogenetic variation in the Slovenian genomic database. Front Pharmacol. 2019;10:240. doi:10.3389/fphar.2019.00240
13. Zhang B, Lauschke VM. Genetic variability and population diversity of the human SLCO (OATP) transporter family. Pharmacol Res. 2019;139:550–9. doi:10.1016/j.phrs.2018.10.017
14. Wright GE, Carleton B, Hayden MR, Ross CJ. The global spectrum of protein-coding pharmacogenomic diversity. Pharmacogenomics J. 2018;18(1):187–95. doi:10.1038/tpj.2016.77
15. Proietti R, Maranho Neto GA, Kunzova S, Re OL, Ahola-Olli A, Heliste J, et al. Pharmacogenomic profile of a Central European urban random population-Czech population. PLoS ONE. 2023;18(4):e0284386. doi:10.1371/journal.pone.0284386
16. Jmel H, Sarno S, Giuliani C, Boukhalfa W, Abdelhak S, Luiselli D, et al. Genetic diversity of variants involved in drug response among Tunisian and Italian populations toward personalized medicine. Sci Rep. 2024;14(1):5842. doi:10.1038/s41598-024-55239-7
17. Matišić V, Brlek P, Bulić L, Molnar V, Dasović M, Primorac D. Population pharmacogenomics in Croatia: evaluating the PGx allele frequency and the impact of treatment efficiency. Int J Mol Sci. 2023;24(17):13498. doi:10.3390/ijms241713498
18. Perović S, Vujović S, Perović S, Šćepanović A. The role of CYP2C19*2 variant and other factors in clopidogrel resistance in Montenegrin ACS patients. Genet Appl. 2024;8(2). doi:10.31383/ga.vol8iss2ga03
19. Velizarova M, Abedinov P, Svinarov D, Nikolov V, Hristova J. Frequency distribution of CYP2C9 and VKORC1 mutations among Bulgarian patients and their importance for anticoagulant therapy. Clin Lab. 2022;68(12):220213. doi:10.7754/Clin.Lab.2022.220213
20. Dokmanovic L, Urosevic J, Janic D, Jovanovic N, Petrucev B, Tosic N, et al. Analysis of thiopurine S-methyltransferase polymorphism in the population of Serbia and Montenegro and mercaptopurine therapy tolerance in childhood acute lymphoblastic leukemia. Ther Drug Monit. 2006;28(6):800–6. doi:10.1097/01.ftd.0000249947.17676.92
21. Kotur N, Lazic J, Ristivojevic B, Stankovic B, Gasic V, Dokmanovic L, et al. Pharmacogenomic markers of methotrexate response in the consolidation phase of pediatric acute lymphoblastic leukemia treatment. Genes. 2020;11(4):468. doi:10.3390/genes11040468
22. Stankovic B, Dragasevic S, Klaassen K, Kotur N, Drazilov SS, Zukic B, et al. Exploring inflammatory and apoptotic signatures in distinct Crohn's disease phenotypes: way towards molecular stratification of patients and targeted therapy. Pathol Res Pract. 2020;216(6):152945. doi:10.1016/j.prp.2020.152945
23. Jojic N, Urosevic J, Bojic B, Pavlovic S. Determination of thiopurine methyltransferase genotype in the patients with inflammatory bowel disease before and during azathioprine therapy. Arch Gastroenterohepatol. 2003;22(1–2):5–9.
24. Jančić I, Arsenović-Ranin N, Šefik-Bukilica M, Živojinović S, Damjanov N, Spasovski V, et al. 174G/C interleukin-6 gene promoter polymorphism predicts therapeutic response to etanercept in rheumatoid arthritis. Rheumatol Int. 2013;33(6):1481–6. doi:10.1007/s00296-012-2586-y
25. Jančić I, Šefik-Bukilica M, Živojinović S, Damjanov N, Spasovski V, Kotur N, et al. Influence of promoter polymorphisms of the TNF-α (-308G/A) and IL-6 (-174G/C) genes on therapeutic response to etanercept in rheumatoid arthritis. J Med Biochem. 2015;34(4):414–21. doi:10.2478/jomb-2014-0060
26. Gemovic B, Veljkovic N. The genomic ecosystem of Serbia situation analysis. Centre fourth industrial revolut Serbia. Available from: https://c4ir.rs/wp-content/uploads/2023/06/ENG_Genomic-Ecosystem-of-Ser

27. Andjelkovic M, Klaassen K, Skakic A, Marjanovic I, Kravljanac R, Djordjevic M, et al. Characterization of 13 novel genetic variants in genes associated with epilepsy: implications for targeted therapeutic strategies. Mol Diagn Ther. 2024;28(5):645–63. doi:10.1007/s40291-024-00720-2
28. Poplin R, Ruano-Rubio V, DePristo MA, Fennell TJ, Carneiro MO, Van der Auwera GA, et al. Scaling accurate genetic variant discovery to tens of thousands of samples. 2017. doi:10.1101/201178
29. Sangkuhl K, Carrillo MW, Whaley RM, Woon M, Lavertu A, Altman RB, et al. Pharmacogenomics clinical annotation tool (PharmCAT). Clin Pharmacol Ther. 2020;107(1):203–10. doi:10.1002/cpt.1568
30. Lee S-B, Wheeler MM, Thummel KE, Nickerson DA. Calling star alleles with Stargazer in 28 pharmacogenes with whole genome sequences. Clin Pharmacol Ther. 2019;106(6):1328–37. doi:10.1002/cpt.1552
31. Graffelman J. Exploring diallelic genetic markers: the HardyWeinberg package. J Stat Softw. 2015;64(3). doi:10.18637/jss.v064.i03
32. Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. A genomic mutational constraint map using variation in 76,156 human genomes. Nature. 2024;625(7993):92–100. doi:10.1038/s41586-023-06045-0
33. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015;31(21):3555–7. doi:10.1093/bioinformatics/btv402
34. Privé F, Aschard H, Ziyatdinov A, Blum MGB. Efficient analysis of large-scale genome-wide data with two R packages: bigstatsr and bigsnpr. Bioinformatics. 2018;34(16):2781–7. doi:10.1093/bioinformatics/bty185
35. Sherman CA, Claw KG, Lee S-B. Pharmacogenetic analysis of structural variation in the 1000 Genomes Project using whole genome sequences. Sci Rep. 2024;14(1):22774. doi:10.1038/s41598-024-73748-3
36. Kovac MK, Maslac AR, Rakicevic LB, Radojkovic DP. The c.-1639G>A polymorphism of the VKORC1 gene in Serbian population: retrospective study of the variability in response to oral anticoagulant therapy. Blood Coagul Fibrinolysis. 2010;21(6):558–63. doi:10.1097/MBC.0b013e32833c2988
37. Mizzi C, Dalabira E, Kumuthini J, Dzimiri N, Balogh I, Başak N, et al. A European spectrum of pharmacogenomic biomarkers: implications for clinical pharmacogenomics. PLoS ONE. 2016;11(9):e0162866. doi:10.1371/journal.pone.0162866
38. Milosevic G, Kotur N, Krstovski N, Lazic J, Zukic B, Stankovic B, et al. Variants in TPMT, ITPA, ABCC4, and ABCB1 genes as predictors of 6-mercaptopurine induced toxicity in children with acute lymphoblastic leukemia. J Med Biochem. 2018;37(3):320–7. doi:10.1515/jomb-2017-0060
39. Vukovic M, Radlovic N, Lekovic Z, Vucicevic K, Maric N, Kotur N, et al. UGT1A1 (TA)n promoter genotype: diagnostic and population pharmacogenetic marker in Serbia. Balkan J Med Genet. 2018;21(1):59–68. doi:10.2478/bjmg-2018-0012
40. Ng C-S, Hasnat A, Maruf AA, Ahmed MU, Pirmohamed M, Day CP, et al. N-acetyltransferase 2 (NAT2) genotype as a risk factor for development of drug-induced liver injury relating to antituberculosis drug treatment in a mixed-ethnicity patient group. Eur J Clin Pharmacol. 2014;70(9):1079–86. doi:10.1007/s00228-014-1703-0
41. Rhonda M, Niemi M, Ramsey LB, Luzum JA, Tarkiainen EK, Straka RJ, et al. The clinical pharmacogenetics implementation consortium guideline for SLCO1B1, ABCG2, and CYP2C9 genotypes and statin-associated musculoskeletal symptoms. Clin Pharmacol Ther. 2022;111(5):1007–21. doi:10.1002/cpt.2557
42. Radosavljevic M, Strac DS, Jancic J, Samardzic J. The role of pharmacogenetics in personalizing the antidepressant and anxiolytic therapy. Genes. 2023;14(5):1095. doi:10.3390/genes14051095