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ASSOCIATION OF *CYP2C19*2* C.681G>A (RS4244285) LOSS-OF-FUNCTION ALLELE WITH CARDIOVASCULAR DISEASE RISK IN THE KOSOVO POPULATION

Elshani N[1*], Ukella K[1], Staninova Stojovska M[2], Naumovska Z[2], Kurshumliu M[3], Gorani D[4], Kapedanovska Nestorovska A[2]

[*]**Corresponding Author:** Nora Elshani, Glloku te Shelgjet "Veternik" 10000-Prishtinë, Kosova. Phone: +38344601032. E-mail: nora.elshani1@gmail.com; nora.elshani@rezonanca-rks.com.

ABSTRACT

The *CYP2C19*2 c.681G>A (rs4244285)* loss-of-function (*LOF*) allele has been associated with reduced clopidogrel efficacy and increased risk of major adverse cardiovascular events (MACE). PGx-guided treatment, despite the recommendations, is not fully implemented in routine clinical practice. The primary aim of this hybrid retrospective-prospective study was to determine whether identifying *CYP2C19* LOF patients may benefit the antiplatelet drug prescribing decisions made in Kosovo. The study cohort consisted of clopidogrel treated patients presenting at the University Clinical Center in the period from December 2023 to May 2024. To evaluate the correlation between *CYP2C19* LOF and the treatment outcome in a follow-up period of 2 years, we first assessed the *CYP2C19*2* genotype using the Taq Man Real Time PCR method. Among 150 patients, 58 (19.33%) were identified as carriers *CYP2C19*2 LOF* allele. The observed allele distribution was significantly different when compared with the one reported for a healthy Kosovar population (13.03%). *CYP2C19*2* LOF carriers exhibited a 1.6-fold higher probability of developing cardiovascular disease compared to non-carriers, based on allelic and codominant model of statistical analysis (OR=1.60; 95% CI=1.08-2.37; p=0.018 and OR=1.64; 95% CI=1.04-2.57; p=0.031, respectively). The median observation time of follow up was not reached until this analysis was conducted. Our data supports the potential association of the *CYP2C19*2 LOF* allele with an increased risk for CVD in the population of Kosovo. Our data add to the evidence advising careful consideration of *CYP2C19* genetic diversity when recommending PGx-guided clopidogrel therapy, particularly in populations, such the Kosovar, where genetic determinants are not yet fully elucidated.

Keywords: clopidogrel, *CYP2C19*2*, Kosovo population, pharmacogenetics, risk for cardiovascular disease.

INTRODUCTION

Clopidogrel remains a cornerstone in dual antiplatelet therapy for reducing the risk of cardiovascular events in patients with coronary artery disease (CAD), as well as those undergoing percutaneous coronary interventions (PCI). It is particularly beneficial in preventing thrombotic events in patients at risk for or who have experienced acute coronary syndromes (ACS), including myocardial infarction (MI) with or without ST-elevation (STEMI or NSTEMI) or unstable angina [1]. Clinical trials (such as CAPRIE, PLATO, CLARITY-TIMI 28, COMMIT/CCS-2), have consistently demonstrated clopidogrel's efficacy in various settings, including in secondary prevention and acute coronary syndromes. However, these clinical trials also emphasize the emergence of newer antiplatelet agents, such as prasugrel and ticagrelor that may demonstrate superior efficacy in certain clinical scenarios, challenging the traditional use of clopidogrel in some patient groups [2]. Clopidogrel is the only irreversible $P2Y_{12}$ (purinergic P2Y, G-protein coupled 12) inhibitor to have a class I

[1] Faculty of Pharmacy, Alma Mater Europaea Campus College "REZONANCA", Glloku te Shelgjet "Veternik", 10000 Prishtina, Republic of Kosovo

[2] University Ss Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of pharmaceutical chemistry, Majka Tereza 47, 1000 Skopje, Republic of North Macedonia

[3] Prolab Laboratory Diagnostics Center, Ulpiana D2, Muslim Mulliqi 35, 10000 Prishtina, Republic of Kosovo

[4] University Clinical Center of Kosova, J5V6+98V, 10000 Prishtina, Republic of Kosovo

indication in patients with stable CAD undergoing stent implantation and is recommended in those with a contraindication to ticagrelor or prasugrel or those taking an oral anticoagulant [3]. Despite its proven benefits, clopidogrel's effectiveness as a prodrug is influenced by genetic and non-genetic factors, with genetic variations in the *CYP2C19* gene being a key determinant of its therapeutic outcomes. *CYP2C19* genetic variants, particularly loss-of-function (LOF) alleles like *2, *3, *4, and *5, have been shown to affect clopidogrel metabolism significantly. Individuals carrying two LOF-alleles exhibit reduced enzyme activity, leading to lower levels of the active metabolite and diminished antiplatelet effects. This reduction in efficacy has been associated with a 2-4 fold increased risk of ischemic events, stent thrombosis, and major cardiovascular and cerebrovascular events, particularly in high-risk patient populations [4-9]. As a result, genotype-guided clopidogrel therapy has gained attention, with clinical guidelines from the American College of Cardiology (ACC) and American Heart Association (AHA) recommending its consideration, especially for patients with CAD or those undergoing PCI [10-13]. Despite promising results from studies, such as from the POPular Genetics trial [12], conflicting findings from other studies (such as CURE, TRITON-TIMI 38, EAST-AFNET 4, GRAVITAS, ISAR-REACT 5) have raised concerns about the generalizability of genotype-guided therapy [14]. These inconsistencies in the evidence contribute to the challenges in achieving widespread adoption of routine *CYP2C19* testing. The uncertainty surrounding the benefits of genotype-guided therapy has led to divergent recommendations from regulatory bodies, professional societies and pharmacogenetics consortia [14-16]. For example, The U.S. Food and Drug Administration (FDA) highlights reduced clopidogrel effectiveness in individuals with *CYP2C19* LOF-alleles and suggests alternative therapies for poor metabolizers, whereas the European Medicines Agency (EMA) adopts a more cautious approach, advising against co-administration with *CYP2C19* inhibitors but not mandating genetic testing [3]. Similarly, pharmacogenetic guidelines, such as those from the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG), recommend *CYP2C19* genotype-guided antiplatelet therapy, particularly in specific high-risk populations, but these recommendations differ in certain aspects and have not been universally integrated into clinical practice [3, 17, 18]. The ESC's 2020 guidelines for ACS management propose genotype-guided therapy as an alternative to standard dual antiplatelet therapy with prasugrel or ticagrelor but refrain from endorsing routine testing for all PCI patients due to limited evidence. The AHA also calls for more research to establish the role of genotyping in improving clinical outcomes, particularly for ACS patients and secondary stroke prevention.

In addition to *CYP2C19*, other genetic polymorphisms such as those in *CES1, PON1, ABCB1*, and *P2RY12* also influence clopidogrel's pharmacokinetics and pharmacodynamics by affecting drug absorption, metabolism, and platelet receptor activity and further complicating the variability in clopidogrel response [19, 20]. Non-genetic factors, including age, comorbidities (e.g., chronic kidney disease and diabetes mellitus), and drug interactions (co-administration of medications that act as *CYP2C19* substrates, inhibitors, or inducers) also play a significant role in modulating drug efficacy [21, 22]. Beyond cardiovascular outcomes, *CYP2C19*2* variants have been associated with altered susceptibility to gastrointestinal disorders, such as peptic ulcers and gastroesophageal reflux disease, as well as certain neurological conditions, highlighting their multifaceted impact [21, 23, 24]. Inter-ethnic variability in the distribution of *CYP2C19* genotypes adds another layer of complexity. Certain alleles, such as *CYP2C19*2*, are more prevalent in specific populations, with significant differences observed across ethnic groups [25, 26]. The lack of comprehensive data on the intra-ethnic distribution of *CYP2C19* variants, coupled with challenges such as cost-effectiveness, logistical barriers, and limitations in the local healthcare infrastructure, restrict integration of PGx testing into routine clinical practice, particularly in resource-limited or under-researched settings like Kosovo, where the integration of genetic testing into routine clinical practice remains an ongoing problem.

This study aims to assess the frequency of the *CYP2C19*2* genotype in clopidogrel-treated patients with cardiovascular disease indications (CVDI) in Kosovo and evaluate its association with major adverse cardiovascular events (MACE), including cardiovascular death, nonfatal myocardial infarction, stroke, stent thrombosis, and revascularization.

MATERIAL AND METHODS

Study population

The study population for the current hybrid observational study with retrospective data collection and prospective follow up was comprised of 150 adult patients, 18 years and older, presenting at the cardiology clinic at the University Clinical Center of Kosovo in the period from December 2023 to May 2024. Major inclusion criteria were the use of clopidogrel therapy because of established cardiovascular diseases (CVD), coronary heart disease (CHD), coronary artery disease (CAD), and cerebrovascular disease, particularly patients with acute coronary syndromes (ACS), including myocardial

infarction (MI) with or without ST-elevation (STEMI or NSTEMI) or unstable angina undergoing percutaneous coronary intervention (PCI) with or without stent. Patients were excluded from the study if they had: 1) a prior history of bleeding (e.g., peptic ulcer, intracranial hemorrhage, menstrual bleeding), 2) clinically significant abnormalities in platelet function or severe hepatic insufficiency, 3) drug addiction or alcohol use disorder, or 4) had donated blood within the last 2 months before starting clopidogrel therapy. Female patients on hormone replacement therapy or using an intrauterine contraceptive device were also excluded. These exclusion criteria align with those in previously conducted Phase 3 randomized, double-blind clinical studies of clopidogrel, ensuring a reliable, homogeneous study population while minimizing the risk of confounding variables and adverse effects. Demographic data (age and gender) and clinical data, including indications for clopidogrel therapy (e.g., acute myocardial infarction, stroke, cardiac catheterization for coronary artery disease, cardiac artery bypass graft surgery, carotid angiography, carotid stenting, carotid endarterectomy, and extremity arteriography), as well as risk factors and co-morbidities (such as heart disease, ischemic heart disease, heart failure, hypertension, diabetes mellitus, stroke, and chronic ischemic heart disease), were manually abstracted from the medical health records (MHR). From each participant previously written informed consent was obtained. The follow-up period for Event-Free Survival (EFS) analysis was 24 months. The study complied with the principles of Helsinki Declaration and was approved from Committee Ethics of Alma Mater Europaea Campus College "REZONANCA" (Protocol No: AD-762/23) and Chamber of Pharmacists of Kosova (No.105).

Genotyping procedures

Genomic DNA was extracted from 2 ml of peripheral blood using the NX-48S Genomic DNA Kit according to the manufacturer's recommended protocol (Genolution Inc, Seoul, Republic of Korea) and stored at -20°C until further analysis. DNA purity and concentrations were verified by UV absorption at 260/280 nm using the NanoDrop 2000™ spectrophotometer (Thermo Fisher Scientific). *CYP2C19*2* c.681G>A (rs4244285) polymorphism was genotyped by allele discrimination PCR on a Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA, USA) real-time PCR system using TaqMan® DME genotyping assay (C__25986767_70; Thermo Fisher Scientific). The genotypes were determined in a reaction mix containing 20 ng DNA in a total volume of 25 µL according to the manufacturer's recommended protocol. Positive and negative controls were included on each plate and reproducibility was checked by re-genotyping 15% of the cases. In accordance with the Clinical Pharmacogenetics Implementation Consortium (CPIC)–recommended genotype-to-phenotype classifications, *CYP2C19*2* metabolizer phenotypes were assigned and reported as follows: Poor Metabolizer (PM with 2 no function alleles; **2/*2* genotype), Intermediate Metabolizer (IM with 1 no function allele; **1/*2* genotype), normal metabolizer (NM; **1/*1* genotype) [16]. All genotyping procedures were performed at the Center for Biomolecular Pharmaceutical Analysis, Faculty of Pharmacy-University Ss. Cyril and Methodius, Skopje, Republic of North Macedonia.

Statistical analysis

The study population was analyzed descriptively, with demographic, clinical, and genetic data presented as counts and frequencies (percentages). Allele and genotype frequencies were assessed for Hardy-Weinberg equilibrium using the Chi-squared (χ^2) test. *CYP2C19*2* data from patients were compared with a historical genotype control of the healthy Kosovo population as reported by Krasniqi et al. (2017) [27] utilizing Fisher's exact test. Stratified analyses examined the association between patients *CYP2C19*2* status and risk factors. Odds ratios (OR) with 95% confidence intervals (CI) were calculated, with statistical significance set at $p \leq 0.05$. All analyses were conducted using MedCalc Software v22.026 (Med Calc Software Ltd, Ostend, Belgium).

RESULTS

Baseline characteristics of the 150 Kosovo patients treated with clopidogrel are summarized in Table 1. The mean age was 68 years, 78.7% of patients being older than 61 year and 40% being female. Among the patients, 43.3% presented with an ACS indication (STEMI/NSTEMI). All patients had at least one comorbidity including common conditions such as hypertension, diabetes, and dyslipidemia. Most patients had arterial hypertension (67.3%) and 35.3% of patients presented with more than one comorbidity.

Allele, genotype, and phenotype frequencies of *CYP2C19*2* polymorphism are detailed in Table 2. A total of 34.0% of patients were *CYP2C19* intermediate or poor metabolizers (29.33% IMs and 4.67% PMs). The frequency of *CYP2C19*2* LOF allele was 19.33%. The observed genotype distributions did not significantly deviate from Hardy–Weinberg equilibrium (χ^2=0.532; p=0.465).

Table 3 provides a comparison of *CYP2C19*2* allele and genotype frequencies observed in our cohort of patients with those reported for the healthy Kosovo population. According to the results, *CYP2C19*2 LOF* allele carriers have approximately 1.6 times higher probability

Table 1. Demographic and Clinical Characteristics of the Study Patient Cohort.

		Full cohort N=150	Frequency
Demographic parameters		n	(%)
Age group	< 40 years	2	1.3
	41-60 years	30	20.0
	> 61 years	118	78.7
Gender	Male	90	60.0
	Female	60	40.0
Clinical parameters			
Indication for clopidogrel therapy	STEMI/NSTEMI*	65	43.3
	Other	85	56.7
Co-morbidities	One	97	64.7
	More than one	53	35.3
Diabetes mellitus	With	65	43.3
	Without	85	56.7
Hypertension	With	101	67.3
	Without	49	32.7
Dyslipidemia	With	6	4.0
	Without	144	96.0

* STEMI/NSTEMI - ST segment elevated myocardial infarction / NonST segment elevated myocardial infarction

Table 2. Distribution of the CYP2C19*2 Allele and Genotype/Phenotype Frequencies in the Patient Population.

CYP2C19*2 polymorphism [rs4244285]	**Patient cohort (N=150)**	**Frequency (%)**	
	n	***observed***	***expected***
Genotype (phenotype)#			
***1/*1 (NM)**	99	66.0	65.07
***1/*2 (IM)**	44	29.33	31.19
***2/*2 (PM)**	7	4.67	3.74
Allele			
***1**	242	80.67	*NA*
***2**	58	19.33	

*1/*1 (NM) – Normal Metabolizer; *1/*2 (IM) – Intermediate Metabolizer; *2/*2 (PM) – Poor Metabolizer; NA- non applicable

Table 3. Association of CYP2C19*2 polymorphism and risk for CVD in Kosovo population.

Model of Statistical Analysis	**Patients (N=150)**		**Healthy population# (N=234)**		OR	95 % CI	p- value
	n	Frequency (%)	n	Frequency (%)			
Co – dominant							
*1/*1 (NM)	99	66	178	76.07	1.00		
*1/*2 (IM)	44	29.33	51	21.79	1.295	0.991 - 1.694	0.067
*2/*2 (PM)	7	4.67	5	2.14	1.632	0.896 - 2.701	0.112
Dominant							
*1/*1(NM)	99	66	178	76.07	1.00		
*2/*2+*1/*2 (PM+IM)	51	34	56	23.93	1.334	1.035 - 1.719	0.031
Allelic							
*1 allele	242	80.67	407	86.97	1.00		
*2 allele	58	19.33	61	13.03	1.307	1.06 - 1.612	0.018

Historical genotype control group according to Krasniqi et al., 2017 [27]

Table 4. Distribution of CYP2C19*2 Allele, Genotype, and Phenotype Frequencies in the Patient Population (N=150), According to Age, Gender, and the Most Common CVD Risk Factors.

CYP2C19*2 polymorphism [rs4244285]		Genotype (phenotype)[#]			Allele	
		*1/*1 n (%)	*1/*2 n (%)	*2/*2 n (%)	*2 n (%)	*1 n (%)
Group age	< 40 years	1 (50)	1 (50)	0 (0)	1 (25)	3 (75)
	41-60 years	18 (60)	10 (33.33)	2 (6.67)	14 (23.33)	46 (76.67)
	> 61 years	80 (67.80)	33 (27.96)	5 (4.24)	43 (18.22)	193 (81.78)
Gender	Male	61 (67.78)	25 (27.78)	4 (4.44)	33 (18.33)	147 (81.67)
	Female	38 (63.33)	19 (31.67)	3 (5)	25 (20.83)	95 (79.17)
Diabetes mellitus	With	45 (69.23)	16 (24.62)	4 (6.15)	24 (18.46)	106 (81.54)
	Without	54 (63.53)	28 (32.94)	3 (3.53)	34 (20)	136 (80)
Hypertension	With	67 (66.34)	29 (28.71)	5 (4.95)	39 (19.31)	163 (80.69)
	Without	32 (65.31)	15 (30.61)	2 (4.08)	19 (19.39)	79 (80.61)
Dyslipidemia	With	4 (66.67)	2 (33.33)	0 (0)	16 (61.54)	10 (38.46)
	Without	95 (65.97)	42 (29.16)	7 (4.87)	42 (15.33)	232 (84.67)

*1/*1 (NM) – Normal Metabolizer; *1/*2 (IM) – Intermediate Metabolizer; *2/*2 (PM) – Poor Metabolizer.
All p-values were greater than 0.05, indicating no statistically significant differences.

for developing CVD compared to non-carriers (OR=1.6; 95% CI=1.08-2.37; p=0.018). The association between *CYP2C19*2* allele and increased probability of developing CVD was further confirmed in dominant (NM vs. IM+PM) model of statistical analysis (OR=1.64; 95% CI=1.04-2.57; p=0.031) as well. Additionally, differences in the genotype/phenotype distribution were observed between the patient and historical healthy control groups (66.0%, 29.33% and 4.67% vs 76.07%, 21.76% and 2.14% for NM, IM and PM, respectively), although these differences did not reach statistical significance (codominant analysis: p=0.067 IM, p=0.112 PM; NM as reference). The present analysis does not allow a stratified assessment of association based on age and gender, since it is based on previously published data concerning *CYP2C19*2 LOF* allele in healthy Kosovo population. No statistically significant difference between stratified groups of patients (according to indication for clopidogrel treatment, coexistence of one or more risk factors) with respect to a *CYP2C19*2* variant allele (Table 4; all p>0.05). The median observation time of follow up was not reached until this analysis was conducted.

DISCUSSION

The worldwide implementation of pharmacogenetics highlighted population-specific differences in allelic and genotype/phenotype frequencies of genes coding for drug-metabolizing enzymes. One significant challenge in translating treatment-associated polymorphisms into routine clinical use is the lack of knowledge regarding its frequency in the targeted population in comparison to the population frequency. In the context of clopidogrel treatment, understanding the prevalence of *CYP2C19 LOF* variants within a population is critical for assessing their clinical implications. Careful consideration is necessary when interpreting studies on the association between *CYP2C19* metabolizer phenotype and clopidogrel treatment outcomes. Evidence supporting this association primarily stems from studies involving ACS patients (with at least 50% undergoing PCI) and settings where clopidogrel was compared with an alternative $P2Y_{12}$ inhibitor [28]. Conversely, studies opposing this association often focus on lower risk, non-PCI patients or data that did not strongly justify the utility of PGx guided clopidogrel treatment for secondary stroke prevention or ACS patients [13, 16]. The presented study primarily aimed to determine the frequency of *CYP2C19*2 LOF* allele in clopidogrel treated patients with cardiovascular disease indications (CVDI) and other concurrent risk factors in Kosovo and to evaluate the prognostic value and association of *CYP2C19* PM phenotype with the risk of MACE in this cohort during two-year term follow-up under routine clinical care. The prevalence of the *CYP2C19*2* allele in this cohort of Kosovo patients (19%) and the historical genotype control group of healthy population (13%) was consistent with the range reported in other European populations [29, 30], with latter falling within the 9-18% range observed across the population from the Balkan region, including Turkey (12%), Greece (13%), Macedonia (14.4%), Serbia (11%), Republic of Srpska (16%) in Bosnia and Hercegovina) Croatia (15%) and Slovenia (16%). Intra-population comparisons, however revealed a significantly higher frequency of the *CYP2C19*2 LOF* allele in the patient cohort compared to the healthy Kosovo population reported by Krasniqi et al. (2017), suggesting a possible association between the *CYP2C19*2 LOF* allele and increased overall

probability of CVD. This association appeared to be independent of the type and number of comorbidities as well as patients' previous history on MI (STEMI or NSTEMI). Notably, the proportion of poor metabolizers (PMs) identified in our patient cohort was significantly higher than the reported in studies of other European populations (4.67% vs. 2.4%, respectively) [11, 29, 31].

This study is the first to report this correlation within the Balkan population. Comparable research conducted in Macedonia [32], Serbia [33] and Croatia [34] has demonstrated that *CYP2C19*2* functions as an independent risk factor for adverse treatment outcomes, defined by the occurrence of MACE in patients receiving dual antiplatelet therapy with aspirin and clopidogrel. Notably, this is the first investigation that explores the relationship between the *CYP2C19*2* allele and the risk of CVD among the Balkan population. The potential mechanism by which *CYP2C19*2* may contribute to an increased risk of cardiovascular disease (CVD) extends beyond its pharmacogenetic role, encompassing disruptions in the metabolism of critical endogenous substrates, such as eicosanoids (arachidonic acid derivatives) and steroids. These substrates are integral to maintaining vascular homeostasis, modulating inflammatory processes, and regulating oxidative stress. Impaired CYP2C19 activity could alter the metabolism of these molecules, leading to endothelial dysfunction, compromised vascular function, and dysregulated blood pressure. Such metabolic disruptions may exacerbate conditions like hypertension and atherosclerosis, ultimately contributing to the pathophysiology of CVD [35, 36].

These preliminary findings highlight the importance of investigating intra-population genetic variations to identify specific genetic markers or health risks prevalent within a particular ethnic group. They supplement the growing body of evidence linking the *CYP2C19*2 LOF* allele with the occurrence and development of CAD, CVD and CHD. The results align with previously published studies suggesting that the *CYP2C19 LOF* allele may be involved in CVD susceptibility [37-41]. Rothenbacher et al. (2013) reported that stable CHD patients, carriers of the homozygous *CYP2C19*2 LOF* allele are at increased risk for subsequent CVD events during the long follow up, independent of other risk factors [37]. Similarly, Zhang et al. (2019) found that *CYP2C19*2* not only increased the risk of CHD, but also worsened clinical outcomes in CHD patients during an extended follow-up period [38]. Cai et al. (2023) observed that among the Hakka population, carriers of the *CYP2C19 LOF* alleles, both heterozygous and homozygous, exhibited increased susceptibility to hypertension [39]. In Martínez-Quintana's study (2017), patients with an acute coronary event and poor or normal CYP2C19 metabolizer phenotype were more likely to have insulin-dependent diabetes mellitus than those with rapid or ultrarapid metabolizer phenotypes [40]. More recently, Chen et al. (2024) reported that carriers of *CYP2C19 LOF* alleles are at increased risk for premature coronary artery disease, particularly when combined with other risk factors such as overweight, smoking, hypertension and diabetes mellitus [41].

When addressing the observed intra-population differences in *CYP2C19*2* frequencies, it is important to acknowledge that relying on previously published data may introduce potential variability due to differences in study design, methodology, or population characteristics and recruitment between historical genotype controls and the study cohort might affect the validity of direct comparisons. However, we recognize this limitation arising from the lack of a contemporaneously recruited healthy control group, as well as the small sample size and absence of demographic data for the healthy population. Despite these limitations, the internal validity of our findings remains intact. Importantly, there were no differences in the sample collection, handling and storing, DNA extraction or genotyping procedures, and both studies used DNA extraction kits comparable in terms of sensitivity and specificity and employed the same PCR-based methods and genotyping assays. Additionally, both the patient cohort and the historical control group primarily consist of ethnic Albanians from all parts of Kosovo. Nevertheless, it is essential to consider that differences in exposure to environmental factors, diet, or healthcare access between the cohorts could act as confounding variables, potentially masking genetic associations. Overall, these results provide valuable insights into the allele, genotype, and phenotype frequencies of the *CYP2C19* drug-metabolizing enzyme in Kosovar patients indicated for clopidogrel treatment. They add to the growing evidence advising the need for careful consideration of *CYP2C19* genetic diversity as a population-specific factor when recommending PGx-guided clopidogrel therapy [25, 26], particularly in populations like Kosovo's, where genetic determinants are not yet fully elucidated. The observed association between the *CYP2C19*2 LOF* allele and increased probability of developing CVD for clopidogrel treatment in our study hinders the ability to conclusively demonstrate a clinical benefit of *CYP2C19*2* PGx guided treatment in the population of Kosovo. Variability in treatment outcome, if identified during the study follow up period, could potentially be attributed to additional patient-specific determinants (such as comorbidities, concomitant drug treatments or as-yet undiscovered population-specific genetic factors). Future studies incorporating a broader range of demographic and clinical variables are needed to better elucidate the role of *CYP2C19*2* PGx guided therapy in optimizing clopi-

dogrel treatment strategies for this population. Moreover, additional investigations should evaluate whether the carriage of the *CYP2C19*2* allele itself is associated with an adverse outcome in patients not taking clopidogrel.

CONCLUSION

This study identifies a possible association between the *CYP2C19*2* allele and an increased probability of CVD within the Kosovo population, offering novel insights into the intra-ethnic variability of this LOF allele and its clinical relevance for PGx-guided therapy within the region. The findings should be considered when optimizing the implementation of the clopidogrel PGx testing in the routine clinical practice at the national level. Further studies examining the relationship between the *CYP2C19* PGx data, patient-specific follow up outcomes, and plasma concentration of clopidogrel's active metabolite are necessary to validate these observations and assess the utility of PGx-guided clopidogrel treatment in Kosovo population.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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