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Original article

GENETIC POLYMORPHISMS OF *CYP2C9* GENE AMONG VOLUNTARY DONORS FROM THE MACEDONIAN BONE MARROW DONOR REGISTRY AND ITS IMPLICATIONS ON THERAPEUTIC DOSAGE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Abstract

The CYP2C9 gene is related to the metabolism of several non-steroidal anti-inflammatory drugs (NSAID). Presence of different allele polymorphisms is associated with lack of enzyme activity and can lead to therapeutic failure. The aim of our study was to provide data about the distribution of CYP2C9 alleles in voluntary donors from the Macedonian Bone Marrow Donor Registry (MBMDR) and to calculate the activation score (AS) of CYP2C9 enzyme.

We analyzed samples of 25 voluntary bone marrow donors by multiplex real-time polymerase chain reaction (PCR) test for detection of CYP2C9*2 and *3 polymorphisms. The samples were analyzed with 7500 Real-Time PCR System with FAM, VIC, ROX and Cy5 filters.

We identified 13 donors with *1*1 diplotype, 7 with *1*3 diplotype, 4 with *1*2 diplotype and 1 donor with *2*3 diplotype. According to the calculated AS, 13 out of 25 individuals (54%) had normal AS value of 2, which refers to normal metabolizers (NMs). Eleven donors (44%) had AS value of 1-1.5, which refers to intermediate metabolizers (IMs) and only one donor (4%) had AS value of 0.5 which refers to poor metabolizer (PM).

Even though the number of donors in our study is small and the results need to be evaluated in a larger cohort, it shows that different genotypes in the CYP2C9 gene are present among donors from MBMR. It is important to have it in mind especially when using NSAIDs that can be bought over-the-counter.

Keywords: pharmacogenetics, CYP2C9, NSAIDs, Macedonian Bone Marrow Donor Registry

Introduction

The CYP2C9 gene, located on the 10q23.33 chromosome, takes part of a cluster of four CYP2C genes along with CYP2C8, CYP2C19, and CYP2C18 [1,2]. This 9 exon gene codes for a CYP2C9 enzyme which is a member of the major cytochrome P450 superfamily (CYP450) of hepatic microsomal enzymes responsible for metabolizing diverse physiologically bioactive substances, medications, and environmental toxins[1-3]. Around 15% of clinically used medications are metabolized by CYP2C9 enzyme, such as warfarin, tolbutamide, glipizide, phenytoin, cyclophosphamide, losartan, and various NSAID (celecoxib, diclofenac, flurbiprofen,

indomethacin, ibuprofen, lornoxicam, meloxicam, nabumetone, piroxicam, and tenoxicam) [1,3,4]. More than 60 allele variations of the highly polymorphic CYP2C9 gene have been identified other than the normal "wild-type" gene allele (***CYP2C9*1***), each of them labeled by a star (*) allele nomenclature[3,4]. Besides the normal gene allele, ***CYP2C9*2*** (3608C>T; p.Arg144Cys; rs1799853) and ***CYP2C9*3*** (42614A>C; p.Ile359Leu; rs1057910) are the most commonly reported polymorphisms associated with a lack of enzyme activity among Europeans, with an allele frequency of 12.28% and 6.67%, respectively [3,5]. CYP2C9*2 and *3 polymorphisms are defined by a single nucleotide substitution 3608C>T in exon 3 and 42614A>C in exon 7, respectively resulting in decreased enzyme activity in CYP2C9*2 and no enzyme activity in CYP2C9*3[4,6]. If we obtain the patient's allele combination, often referred to as diplotype, we can calculate the CYP2C9 enzyme activation score (AS) by summation of the allele's functional values. The allele functional status extends from 0 to 1 (0 for no function; 0.5 for decreased function; 1 for normal function). Therefore, we can stratify patients into three groups: **poor metabolizers** (with AS varying from 0-0.5); **intermediate metabolizers** (with AS varying from 1-1.5) and **normal metabolizers** (with AS equals 2)[4] (Table 1**)**.

Table 1. Stratification of different genotypes according to phenotype and CYP2C9 activation score (AS)[6]

Likely phenotype	Activation score	Genotypes and examples of diplotypes
Normal metabolizers (NMs)	2	*CYP2C9*1*1*
Intermediate metabolizers (IMs)	1.5	*CYP2C9*1*2*
	1	*CYP2C9*1*3/ *2*2*
Poor metabolizer (PMs)	0.5	*CYP2C9*2*3*
	0	*CYP2C9*3*3*

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics to treat acute pain, dysmenorrhea, fever, inflammation, and rheumatic diseases such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and gout[3,7,8]. Pain relief is achieved by inhibiting the cyclooxygenase enzyme (COX) and therefore suppressing prostaglandins, prostacyclin and thromboxane biosynthesis from arachidonic acid[3,4]. COX-1 and COX-2 are the two main isoforms of the cyclooxygenase enzyme[3]. Even though NSAIDs are considered safe, their usage is responsible for up to 30% of hospitalizations due to adverse drug reactions (ADRs) such as gastrointestinal bleeding, ulceration, and myocardial infarction[3,8]. High-risk patients for the occurrence of ADRs are those who are older than 65 years, those who have diabetes, high blood pressure, kidney or liver damage, a prior episode of gastrointestinal hemorrhage, and patients on prolonged NSAIDs treatment[8]. Evidence shows that the most NSAID therapeutic failures are dose-dependent, which could be caused by decreased or no function of the CYP2C9 enzyme as a result of CYP2C9 genotype[4].

The aim of our study was to detect the distribution of different CYP2C9 alleles among voluntary donors from Macedonian Bone Marrow Donor Registry (MBMDR) and to compare them to previously published data for Macedonians, European data and other neighboring countries.

Materials and methods

Study group

In our study we included 25 voluntary donors randomly selected from the Macedonian Bone Marrow Donor Registry, at the Institute of Immunobiology and Human Genetics at the

Faculty of Medicine, Ss. Cyril and Methodius University in Skopje. The study population consisted of 11 men (44%) and 14 women (56%), aged between 21 and 73 years (mean age 36.28 ±12.78).

Methods

The identification of the CYP2C9 diplotype for each of the 25 donors was done with a multiplex real-time PCR test for the simultaneous detection of CYP2C9*2 and *3 polymorphisms of the human CYP2C9 gene, produced by the manufacturer Vienna Lab Diagnostics GmbH, Austria.

This commercial kit supplies Probe Mix, Assay Mix and two positive controls for CYP2C9*1*1 and *2*3. The analysis was conducted according to the manufacturer's instructions. Signed informed consent was obtained from all 25 donors. DNA was isolated from peripheral blood and real time-PCR was performed. The analysis was performed on 7500 Real-Time PCR System, Applied Biosystems, Thermo Fisher, USA. The PCR products were detected using blue filter-FAM (520 nm), green filter-VIC (556 nm), red filter-ROX (605 nm), and pink filter-Cy5 (670 nm), followed by interpretation of the results according to the manufacturer's instructions.

Statistical analysis

Descriptive statistics of the results was performed. For comparison, we used chi-square test (χ^2 test), and $p < 0.05$ was considered statistically significant.

Results and discussion

Figure 1 shows the amplification of different CYP2C9 alleles detected by FAM, VIC,

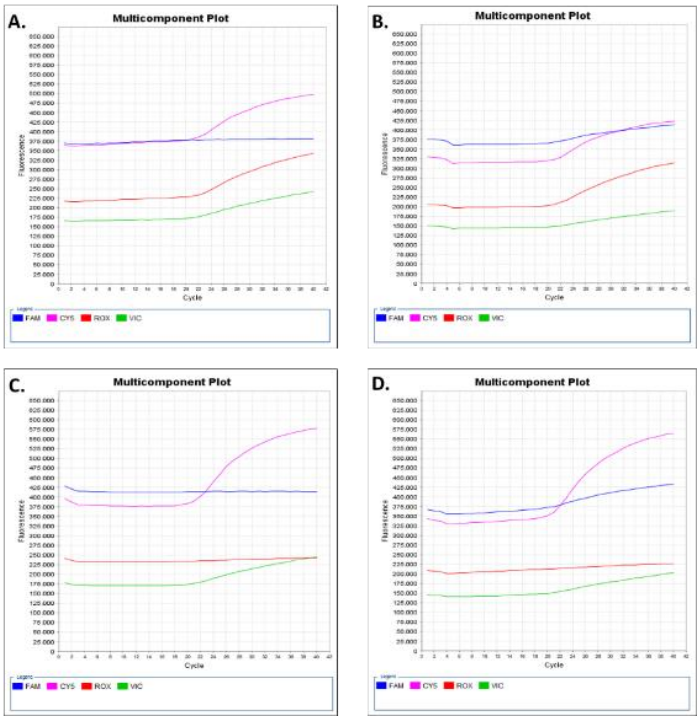

Fig. 1. Examples from the results obtained after the run of the multiplex real-time PCR for simultaneous detection of CYP2C9*2 and *3. **A.** Amplification of CY5, ROX, VIC (CYP2C9*1*3); **B.** Amplification of FAM, CY5, ROX, VIC (CYP2C9*2*3); **C.** Amplification of CY5, VIC (CYP2C9*1*1); **D.** Amplification of FAM, CY5, VIC (CYP2C9*1*2)

ROX, and Cy5 filters. The highest allele frequency in our group was obtained for CYP2C9*1 (74%), followed by CYP2C9*2 and CYP2C9*3 alleles with 10% and 16%, respectively (Figure 2A). In this study, normal metabolizers who carry CYP2C9*1*1 diplotype were predominant - 13 donors out of 25 (52%) with an activation score of the CYP2C9 enzyme of 2. The NMs were followed by the group of intermediate metabolizers (IMs) with an activation score varying from 1-1.5. The group of IMs comprised 7 donors (28%) who carried CYP2C9*1*3 with an activation score of 1, and 4 donors (16%) who carried CYP2C9*1*2 with an activation score of 1.5. Only one donor (4%) who carried CYP2C9*2*3 diplotype was a poor metabolizer with an activation score of 0.5 (Figure 2B).

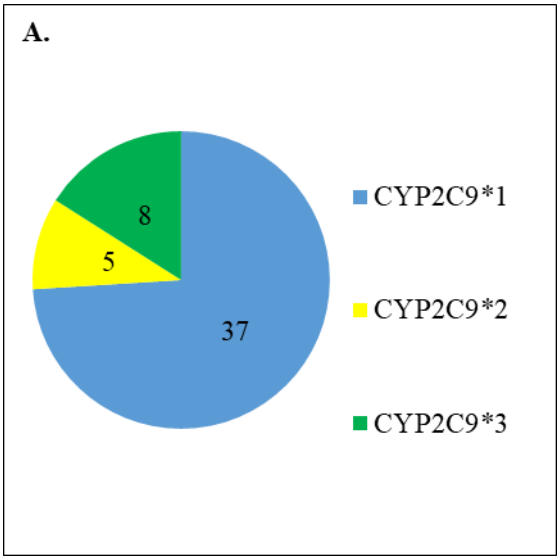

Fig 2A. Frequency of CYP2C9*1, *2, and *3 alleles detected in voluntary donors from MBMDR.

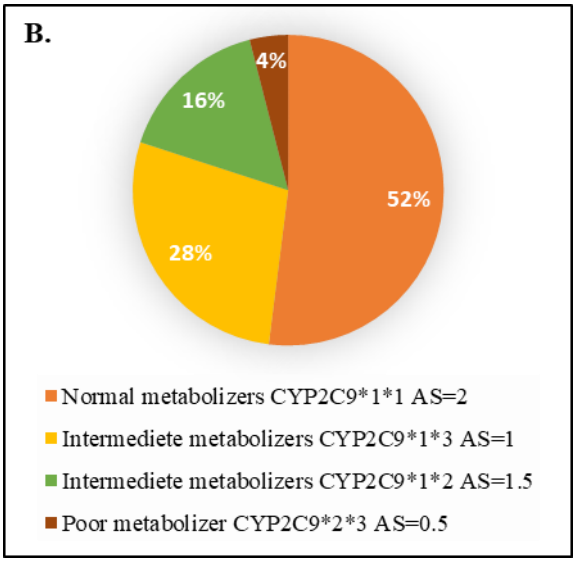

Fig. 2B. The distribution of different types of metabolizers in donors from MBMDR

In our study, NMs with CYP2C9*1*1 genotype were the most prevalent (52%), which was similar to the data previously reported for Macedonians (57.8%)[10]. The prevalence of NMs in Serbians (62.03%), Croatians (59.72%) and Greeks (62.19%) is slightly higher compared to Macedonians[11-13]. IMs comprise 44% of our study population, out of which 28% have CYP2C9*1*3 genotype and the rest 16% have CYP2C9*1*2 genotype. The frequency of IMs we obtained was much higher in Macedonians than in Serbians (36.11%). As for CYP2C9*1*2 carriers in our study, the percentage was slightly decreased compared to previously published data for Macedonians (19.6%), Serbians (21.76%), Croatians (23.52%), and Greeks (20.14%) [10-13]. On the other hand, the frequency of CYP2C9*1*3 carriers in this study was much higher compared to earlier published data for Macedonians (10.3%), Serbians (12.04%), Croatians (12.78%), and Greeks (13.43%)[10-13]. In our study population, we detected only one PMs with CYP2C9*2*3 genotype (4%) as expected from the previously published data for Macedonians (1%) and Serbians (0.93%)[10,11]. According to the published data, PMs are more prevalent in Croatians and Greeks where they account for 1.94% and 1.41% of all types of metabolizers, respectively[12,13]. A more detailed comparison between different nationalities is presented in Table 2. None of these differences reached a statistical significance (p=0.534477). We did not detect CYP2C9*2*2 and CYP2C9*3*3 genotypes in our study group, probably due to the small sample size.

Table 2. CYP2C9 genotype distribution among donors from MBMDR, Macedonian, Serbian, Croatian, and Greek populations [10-13]

Genotype	Donors from MBMDR (this study)	Macedonians (Jakovski *et al*. 2013)	Serbians (Vidović *et al*. 2021)	Croatians (Ganoci *et al*. 2017)	Greeks (Arvanitidis *et al*. 2007)	P value
CYP2C9*1*1	n= 13 (52%)	n= 112 (57.8%)	n= 134 (62.03%)	n= 645 (59.72%)	n= 176 (62.19%)	p=0.534477
CYP2C9*1*2	n= 4 (16%)	n= 38 (19.6%)	n= 47 (21.76%)	n= 254 (23.52%)	n= 57 (20.14%)	
CYP2C9*1*3	n=7 (28%)	n= 20 (10.3%)	n= 26 (12.04%)	n= 138 (12.78%)	n= 38 (13.43%)	
CYP2C9*2*3	n=1 (4%)	n= 2 (1%)	n= 2 (0.93%)	n= 21 (1.94%)	n= 8 (2.21%)	
CYP2C9*2*2	/	n= 5 (2.6%)	n= 5 (2.31%)	n= 19 (1.76%)	n= 4 (1.41%)	
CYP2C9*3*3	/	n= 2 (1%)	n= 2 (0.93%)	n= 3 (0.28%)	/	
Total in of cases	25	179	216	1080	283	

(n- Number of participants)

Based on the scientific evidence to date, which proves the correlation between CYP2C9 genotype and ADRs during NSAID treatment, the Clinical Pharmacogenetics Implementation Consortium (CPIC) established therapeutic recommendations towards NSAID dosage[4]. In terms of ibuprofen, a nonselective COX inhibitor with half-life (t½=2-4 hours), the CPIC suggests for NMs with AS=2 and IMs with AS=1.5 to start the treatment with the approved daily dose [4,9]. On the other hand, for IMs with AS= 1, it is recommended to begin the treatment with the lowest recommended dose, due to a greater elimination half-life up to 47%[4,9]. As for PMs with AS=0-0.5, the dosage of ibuprofen should be decreased by 25-50% of the recommended starting dose

or even consider alternative CYP2C9-unassociated drugs, such as aspirin, ketorolac, naproxen, and sulindac[4].

In conclusion, our study shows that different genotypes of the CYP2C9 gene are present in the donors from MBMDR, in addition to their polymorphic HLA profile. However, the number of participants in our study was small and we were not able to detect the polymorphisms that have lower frequencies. Nevertheless, NSAIDs are one of the most used over-the-counter drugs and different types of drug metabolizers have to be kept in mind in order to obtain an effective drug dose and to decrease the appearance of adverse drug reactions in patients.

Conflict of interest statement. None declared.

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