BMC Genomics

RESEARCH

Open Access

Frequencies of CYP2C9 polymorphisms in a Syrian cohort

Weam Aldiban[1,2], Majd N. Aljamali[3,4] and Lama A. Youssef[1,2,3*]

Abstract

Background The cytochrome P450 family 2 subfamily C member 9 (CYP2C9) exhibits extensive genetic variability that may influence the metabolism of approximately 16–20% of all drugs. Understanding the frequency and functional impact of the *CYP2C9* variants is crucial for the implementation of pharmacogenetics. Our study aims to determine the frequencies of CYP2C9 variants in the Syrian population, contributing to the limited information available for Middle Eastern populations.

Methods One hundred thirty-eight unrelated individuals from two major Syrian cities (Damascus and Homs) enrolled in this cross-sectional study. Genomic DNA was extracted from peripheral blood and specific PCR amplification products were purified and sequenced. The length of the amplicons allowed for the detection of 17 star alleles (i.e. **2*, **8*, **14*, **20*, **26*, **33*, **40*, **41*, **42*, **43*, **45*, **46*, **62*, **63*, **72*, **73*, and **78*) in exon three, and seven star alleles (i.e., **3*, **4*, **5*, **24*, **55*, **66*, **68*) in exon seven, in addition to two intronic variants. The frequencies of the functionally compromised *CYP2C9*2*^{rs1799853} and *CYP2C9*3*^{rs1057910} alleles were compared to same variants in other populations.

Results Of the 24 exonic alleles investigated, only the **2*, **3*, **41*, and **46* alleles were detected at frequencies of 14.8%, 8.3%, 1.45%, and 0.72%, respectively, with 43.5% of the study subjects carrying at least one dysfunctional variant. The genotype frequencies observed were as follows: **1/*1* (56.5%), **1/*2* (23.9%), **2/*2* (0.7%), **3/*1* (12.3%), **2/*3* (4.3%), **3/*3* (0%), **1/*41* (0.7%), **2/*41* (0%), **3/*41* (0.7%), **1/*46* (0.7%), **46/*2* (0%), and **46/*3* (0%). Moreover, frequencies of the rs933120 and rs933119 intronic alleles were 12.3% and 6.1%, respectively. A high linkage disequilibrium (LD) was found (D'=0.78) between the intronic rs933119 and exonic rs1799853 **(***2 allele).

Conclusions This study provides evidence for high prevalence of the *CYP2C9 *2* and **3* alleles, and consequently the intermediate and poor metabolizer phenotypes in Syrians. Two rare putative function-relevant variants (**41* and **46*) were detected in three individuals. These findings pave the path to the efforts for implementing *CYP2C9* pharmacogenetics-based personalized pharmacotherapy in this Middle Eastern population.

Keywords *CYP2C9*, SNVs, Allele frequency, Pharmacogenetics, Personalized medicine, Syrians

*Correspondence:
Lama A. Youssef
lama.youssef@damascusuniversity.edu.sy; ylama@hotmail.com
[1]Department of Pharmaceutics and Pharmaceutical Technology, Program of Clinical and Hospital Pharmacy, Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic
[2]Faculty of Pharmacy, International University for Science and Technology (IUST), Ghabagheb, Daraa, Syrian Arab Republic
[3]National Commission for Biotechnology (NCBT), Damascus, Syrian Arab Republic
[4]Department of Biochemistry and Microbiology, Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic

Introduction

The liver is a major multi-functional organ that plays a central role in drug metabolism via Phase I and/or phase II reactions. Phase I encompasses complex processes mediated mainly by the cytochrome P450 (CYP450) superfamily enzymes, primarily found in hepatocytes and are involved in the oxidation and reduction reactions to enhance water solubility of most drugs and facilitate their excretion [1–2].

The inter-individual variability regarding response to drugs can be attributed to extrinsic factors (i.e., alcohol consumptions, smoking, nutrition, environmental and drug interactions) as well as intrinsic characteristics (such as age, gender, ethnicity, comorbidities, and genetic makeup) [3–4]. Genetic variations can explain a significant proportion of inter-individual variability in drug pharmacokinetics including drug absorption, metabolism, distribution, and elimination (ADME) and drug pharmacodynamics (drug efficacy and effectiveness) [4].

The individual characteristics of each patient are collected and processed to tailor medical treatment in a medical model known as "personalized medicine" or "precision medicine", which has gained paramount interest in recent years as a systematic approach to maximize therapeutic benefits and minimize adverse drug reactions (ADRs) [3].

One particularly important enzyme in drug metabolism is the CYP2C9, which is highly expressed in the liver and is responsible for the hydroxylation of a significant percentage of drugs, such as S-warfarin, fluoxetine, phenytoin, gliclazide, and nonsteroidal anti-inflammatory drugs (NSAIDs) [5–6]. The efficacy of CYP2C9 can be influenced by various drugs, including inducers, such as rifampicin, carbamazepine, and phenobarbital, as well as inhibitors, like amiodarone, fluconazole and sulphaphenazole [7].

CYP2C9 is encoded by a highly polymorphic gene, located on the long arm of chromosome 22, in the chromosomal region 10q23.33, and consists of nine exons encoding a protein of 490 amino acids. Among at least 85 *CYP2C9* star alleles identified, more than 20 single nucleotide variants (SNVs) were proved to affect the enzymatic activity [8], and therefore can lead to variations in the response to substrate drugs, especially those with narrow therapeutic index [9].

*CYP2C9*1* is the wild type and most prevalent allele, whereas *CYP2C9*2* and **3* alleles are the next most common alleles with frequencies of approximately 13% and 8%, respectively in European and Middle Eastern populations [5]. The **2* allele is characterized by a nonsynonymous single base substitution (430 C > T) in exon 3, leading to arginine replacement with cysteine at position 144 in the encoded protein (R144C). The **3* allele (1075 A > C) is located in the seventh exon, causing a single amino acid change from isoleucine to leucine (I1359L) [10]. *CYP2C9*2* and **3* have been extensively studied due to the resulting phenotypes characterized by decreased enzymatic activity; retaining only 16–20% for *CYP2C9*2* and 4–6%, for *CYP2C9*3*, compared to that of the *CYP2C9*1* allele [11]. Individuals with the *CYP2C9*1/*1* genotype are categorized as "extensive metabolizers", carriers of one low enzymatic activity allele (*CYP2C9*1/*2* or *CYP2C9*1/*3*) are classified as "intermediate metabolizers", whereas "poor metabolizers" (*CYP2C9*2/*2*, *CYP2C9*2/*3* and *CYP2C9*3/*3*) refer to those carrying two functionally compromised alleles [12]. Although *CYP2C9*2* and **3* are the most prominent loss of function or no-function variants in Caucasians, additional *CYP2C9* alleles (*CYP2C9*5*, **6*, **8*, and **11*) have been identified in other populations (i.e., African descendants) at frequencies that surpassed those of **2* and **3* [13]. Some of these genetic variants, such as **4* (1076 T > C) and **5* (1080 C > G), are associated with a decreased enzyme activity, whereas some other recently reported *CYP2C9* alleles, including **6*, **15*, **25*, and **35* were designated as non-functional alleles [14].

Nevertheless, the impact of most other SNVs on enzymatic activity has not yet been determined, as the majority of the reported 85 *CYP2C9* variants are currently classified as variants of uncertain significance (VUS). While accurate variant interpretation and currently well-defined genotype-phenotype assignment have enabled clinicians to improve therapeutic decision-making, there is a lack of information on the functional significance as well as prevalence rates of the majority of these VUS. Impact on functionality and a relatively high prevalence are two key determinants for their clinical relevance [15–16].

The strong *CYP2C9* genotype-phenotype correlation has prompted the Food and Drug Administration (FDA) and other drug regulatory agencies to provide recommendations regarding the safety and effectiveness of several *CYP2C9* drug substrates, including oral-anticoagulants (i.e., Warfarin and others), NSAIDs (i.e., Celecoxib, Flurbiprofen, and Piroxicam), antiepileptics (i.e., Phenytoin), anticancer drugs (i.e., Erdafitinib) and others [17].

CYP2C9 genotyping is particularly relevant for narrow therapeutic index, such as S-warfarin. In vitro and in vivo evidence support *CYP2C9*2* and **3* impair metabolism of S-warfarin by approximately 30–40% and 80–90%, respectively compared to patients with the *CYP2C9*1/*1* genotype [18]. Therefore, subjects homozygous for *CYP2C9*2* or **3*, denoted as poor metabolizers, are at greater risk of bleeding during warfarin therapy, which consequently necessitates dose adjustment [19–20]. Additionally, a contraindication warning is issued for using siponimod in *CYP2C9*3/*3* patients [17].

The inter-ethnic variations in *CYP2C9* SNVs distribution rationalize conducting studies to determine prevalence in different populations and ethnic groups, other than Caucasians, in order to achieve a realistic assessment of the feasibility of pharmacogenetic testing as a key pillar of precision medicine, especially for accurately dosing problematic drugs [21–22]. The Middle East, particularly the Levant countries, encompasses an admixture of multiple ethnicities including Arabs, Kurds, Arameneans, Assyrian, Turks, Circassians and others. Despite this diversity, only a scarce number of studies have explored the prevalence of SNVs in pharmacogenes encoding relevant proteins, including Cytochrome P450, in the populations of this region [23].

Our study aimed to determine the frequencies of the most well characterized pharmacologically important *CYP2C9* allelic variants (**2* and **3*), in addition to other well characterized SNVs and less well-defined function variants in Syrians.

Materials and methods

Subjects

This observational cross-sectional study was approved by the Scientific Research Ethics Committee (Reference Number: 1, issued on April 25th, 2016) at the Faculty of Pharmacy, Damascus University. Written informed consent was obtained from each subject. All participants were unrelated Syrian nationals from two major cities; the Capital Damascus and Homs. Peripheral blood was collected using Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant and stored at -20 C° until use.

Genotyping

The molecular work, except polymerase chain reaction (PCR) products sequencing, was performed in the Department of Pharmaceutical Biotechnology at the National Commission for Biotechnology Laboratories, Damascus, Syria. Genomic DNA was isolated from 3 to 5 ml of peripheral blood samples using Nucleospin ® Blood Quick Pure (Bioke, Netherland) according to the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo®, USA). To verify the quality and conservation of the isolated DNA, horizontal 1.5% agarose gel electrophoresis was performed, followed by ethidium bromide staining (Promega®, USA).

Genotyping for the *CYP2C9* alleles was performed by PCR using a thermal cycler (SENSEQUEST®, Germany), followed by standard Sanger sequencing (Macrogen, South Korea). In brief, the PCR reaction mixture contained 20 ng of genomic DNA and 0.25 µm/l of each primer manufactured by Eurofins (Belgium) and 2X Master Mix (Genedirex®, Taiwan). Primers were designed using MFEPrimer 3.1 (iGeneTech®, China) (primer sequences are shown in Table 1).

PCR conditions were set as follows: for the 690 bp amplicon covering the *CYP2C9*2* and other SNVs in exon 3; initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 45 s (s), annealing at 46 °C for 1 min, and extension at 72 °C for 40 s. For the 105 bp amplicon of *CYP2C9*3*: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45s, annealing at 53 °C for 45s, and extension at 72 °C for 40s. PCR products, together with a DNA size marker (Genedirex®, Taiwan), were separated on 1.5% agarose gels. For samples with secondary amplification products, the relevant bands were cut from the gel and purified using a PCR clean-up and gel extraction kit (GeneDirex®,Taiwan).

Bioinformatics and statistical analyses

Sequencing results were analyzed using Snapgene® and Geneious® software. Statistical analyses were performed using Statistical Package for the Social Sciences)SPSS) edition 25. Frequencies of investigated alleles and genotypes were estimated using the gene counting method. The agreement with the Hardy-Weinberg equilibrium of the observed genotypic distribution for the *CYP2C9* gene was tested using chi-square tests. Statistical significance was set at $P < 0.05$. Chi-Square test was performed to compare alleles frequencies in our study cohort and those reported in other populations. A world map with color gradients was generated to display the geographical distribution of allele frequencies. Additionally, GraphPad Prism® was utilized to create graphs visualizing the frequencies of CYP2C9 phenotypes among populations. Specifically, we employed a gradient color scheme to represent frequency distributions: blue shades indicate low frequencies, green represents below-intermediate frequencies, yellow and orange shades correspond to intermediate frequencies, red indicates above-intermediate frequencies, and purple shades denote high frequencies. Finally, linkage disequilibrium (LD) across *CYP2C9* alleles was detected by Haploview® software (version 4.2).

Results

Our cohort encompassed 138 participants from two major Syrian cities; Damascus ($n = 81$, 58.7%) and Homs ($n = 57$, 41.3%), with males constituting 56.5% of the study subjects. The mean age (± standard deviation) of the participants was 47.5 ± 15.5 years. The demographic characteristics (mean age and males/females composition) of the study subjects from the two cities/governorates were comparable, with p values being $>>0.05$ for all comparisons, implying the homogeneity of the study sample. Moreover, our comparison of the genotype distributions also revealed no statistical differences between these two

Table 1 The primers and positions of the CYP2C9 SNVs evaluated in this study

Amino acids change	SNV/SNP ID (NCBI)	position	Primer sequence (5`-3`)	SNP/SNV
R144C	rs1799853	Exon 3	F: TACAAATACAATGAAAATATCATG	*CYP2C9*2*
R150H	rs7900194	Exon 3	R: CTAACAACCAGGACTCATAATG	*CYP2C9*8*
R125H	rs72558189	Exon 3		*CYP2C9*14*
G70R	rs773630199	Intron 2		*CYP2C9*20*
T130R	rs200965026	Exon 3		*CYP2C9*26*
R132Q	rs200183364	Exon 3		*CYP2C9*33*
F110S	rs559536673	Intron 2		*CYP2C9*40*
K119R	rs774607211	Exon 3		*CYP2C9*41*
R124Q	rs12414460	Exon 3		*CYP2C9*42*
R124W	rs767576260	Exon 3		*CYP2C9*43*
R132W	rs199523631	Exon 3		*CYP2C9*45*
A149T	rs754487195	Exon 3		*CYP2C9*46*
R125C	rs375805362	Exon 3		*CYP2C9*62*
R144H	rs141489852	Exon 3		*CYP2C9*63*
A149V	rs1289704600	Exon 3		*CYP2C9*72*
R150C	rs17847037	Exon 3		*CYP2C9*73*
L128R	rs1375956433	Exon 3		*CYP2C9*78*
--	rs9332119	Intron 1		--
--	rs9332120	Intron 2		--
I359L	rs1057910	Exon 7	F: TGCACGAGGTCCAGAGATAC	*CYP2C9*3*
I359T	rs56165452	Exon 7	R: ACAAACTTACCTTGGGAATGAGA	*CYP2C9*4*
D360E	rs28371686	Exon7		*CYP2C9*5*
E354K	rs749060448	Exon7		*CYP2C9*24*
L361I	rs1250577724	Exon7		*CYP2C9*55*
L362V	rs578144976	Exon7		*CYP2C9*66*
Splice Defect	rs542577750	Exon7		*CYP2C9*68*

subgroups (P value = 0.731) as well as an almost identical distribution of genotypes in males and females (P value = 0.94).

Expectedly, the wild-type *CYP2C9*1* allele was predominant (75.36%), followed by the *CYP2C9*2* (14.8%) and *CYP2C9*3* (8.3%). Surprisingly, two individuals (0.72%) were carriers of one copy of the *CYP2C9*41* allele (NG_008385.2:g.9059 A > G), and one subject (0.36%) was a carrier of one copy of the *CYP2C9*46* allele (NG_008385.2:g.9148G > A). None of the other SNVs were detectable in any of the study subjects. Overall, 56.5% of the patients were genotyped as wild type (*CYP2C9*1*1*), whereas 43.5% had one or two of *CYP2C9*2,*3,*41,*46* alleles. The genotypes and frequencies observed in the study cohort are listed in Table 2.

The observed frequencies of *CYP2C9*2*, *CYP2C9*3*, *CYP2C9*46*, and *CYP2C9*41* alleles were in Hardy-Weinberg equilibrium, with P values of 0.21, 0.33, 0.95, and 0.9, respectively. Two intronic SNVs (rs9332120 in intron 2 and rs9332119 in intron 1) were detected at allelic frequencies of 12.3% and 6.1%, respectively. Noteworthy, a strong association between the intronic rs9332119 allele and the exonic rs1799853 (*CYP2C9*2*) was detected (D'=0.78). The overall linkage disequilibrium across the *CYP2C9* gene alleles is shown in Fig. 1.

Discussion

CYP2C9, an abundant hepatic cytochrome P450 enzymes, is involved in metabolism of 16–20% of all clinically important drugs (such as warfarin, phenytoin, and NSAIDs) [24]. However, *CYP2C9* is highly polymorphic and inter-individual variation in *CYP2C9* expression and activity can lead to variations in drug responses and adverse drug effects in considerably large proportion of patients [7]. Dozens of *CYP2C9* polymorphisms resulting in substantial differences in enzyme activity have been identified, with some may lead to almost complete loss of function. The distribution of these alleles varies significantly among different ethnic groups [25–26]. The genetic makeup and frequency of relevant gene polymorphisms affecting drug response in Middle Easterners and Levantines are still quite under-investigated, and studies on Syrians' are scarce. To the best of our knowledge, this is one of the first studies to investigate the frequency of *CYP2C9* polymorphisms in a Syrian population.

Of the 85 *CYP2C9* variants, *CYP2C9*2* and *CYP2C9*3* have received most attention due to their association with substantially decreased enzymatic activity and widespread prevalence, especially among Europeans and other Middle-Eastern populations [27]. Our findings prove high prevalence of the *CYP2C9*2* and *CYP2C9*3*

Table 2 Frequency distribution of the *CYP2C9* alleles and genotypes with predicted phenotypes in the study cohort

Genotype		Frequency *n* (%)	Predicted Phenotype	Total No (%)
**1/*1*	**Wt/Wt**	78 (56.5)	**Normal/Extensive Metabolizers**	**78 (56.5)**
**1/*2*	**Wt/Var**	33 (23.9)	**Intermediate Metabolizers**	**52 (37.6)**
**1/*3*		17 (12.3)	**Intermediate Metabolizers**	
**1/*46*		1 (0.7)	**Intermediate Metabolizers**	
**1/*41*		1 (0.7)	**Uncertain**	
**2/*2*	**Var/Var**	1 (0.7)	**Poor Metabolizers**	**8 (5.8)**
**2/*3*		6 (4.3)	**Poor Metabolizers**	
**3/*41*		1 (0.7)	**Uncertain**	
Alleles		**Frequency (%)**	**95%CI**	
*CYP2C9*1*		75.36	0.69–0.80	
*CYP2C9*2*		14.8	0.10–0.18	
*CYP2C9*3*		8.3	0.05–0.11	
*CYP2C9*41*		0.36	0.0-0.01	
*CYP2C9*46*		0.72	0.0-0.01	

Frequency for alleles; **4,*5, *8, *14, *20,*24, *26, *33, *40,*42, *43, *45,*55, *62, *63, *66, *68, *72, *73, *78* equals 0

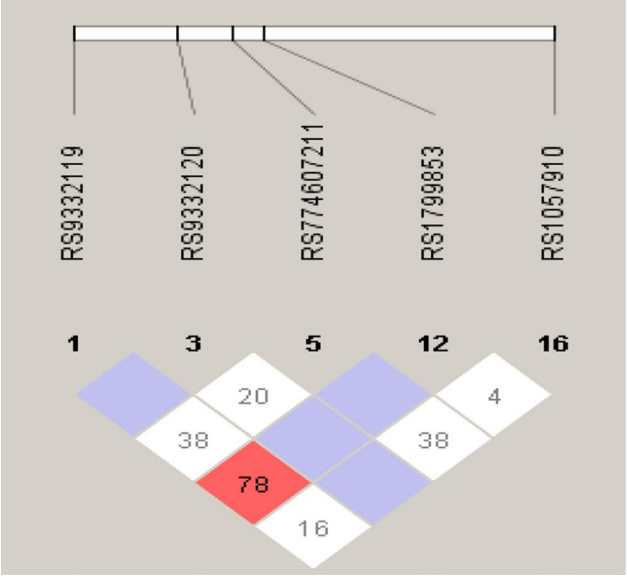

Fig. 1 Linkage disequilibrium plot with the genomic positions indicated at the top. ID'I × 100; ID'I color scheme: ID'I = 0: white; 0 < ID'I < 1: shades of pink; ID'I = 1: red

(14.8% and 8.3%, respectively) in our Syrian cohort, which is almost equivalent to, and even slightly exceeding, the reported prevalence in Caucasians of 11.1–14.4% and 7%, respectively [27].

Our findings prove similarity of the *CYP2C9*2* prevalence (14.8%) in Syrians with those reported in studies conducted in neighboring populations, such as those in Lebanese (15.4%), Palestinians (13.6%) Jordanians (13.5%) and Turks (13%) [28–31], but not with Iranians, where the prevalence *of CYP2C9*2* is significantly higher and reaches 25% [20]. The frequencies *CYP2C9*2*, however, in the Arabian Gulf Countries drop to 7.2% and 8% in the United Arab Emirates (UAE) and Sultanate of Oman, respectively and reach 11.7% in Saudi Arabia [32–34]. Similarly, lower frequencies are observed in North African Arab states, such as Morocco (8%) and Egypt (11.7%) [35–36].

The distribution of *CYP2C9*2* in Southern Europe aligns with that of the Eastern Mediterranean, with reported frequencies of 12.6% and 14.3% in Italy and Spain, respectively [37–38], and a slightly higher frequency of 16% was observed in France [39]. Reports from northern European countries revealed relatively declined frequencies to 11.8%, 11%, and 10.7% in Russia, the United Kingdom, and Sweden, respectively [40–42].

In contrast to the high prevalence *CYP2C9*2* in Europe and the Middle East, substantially lower frequencies are reported in sub-Saharan Africa and East Asia. *CYP2C9*2* is virtually absent in sub-Saharan countries such as Benin, Ghana, and Mozambique (0%) [43–45], and with prevalence rates not exceeding 4.3% in several other African nations, including Ethiopia [46].

South and East Asia also demonstrates low prevalence of *CYP2C9*2* with a reported frequency of only 4.6% in Indians [47], and a nadir of 0.1% in China and 0% in Japan [16, 48].

South and Central American countries exhibit considerably variable *CYP2C9*2* prevalence, with rates ranging from 26% in Argentina to 10.6% and 8% in Brazil and Mexico, respectively. [49–50, 51, 52].

North American populations represent an admixture of ethnicities; however, a comparable frequency to Europeans was reported (14.6%) [51]. A world map illustrating the published *CYP2C9*2* allele frequencies in the Middle East countries, including Syria, and other countries in the five continents is shown in Fig. 2.

The frequency (8.3%) of the *CYP2C9*3* allele in our population was comparable to that found in Caucasians (7.1%), as reported in a plethora of studies that explored

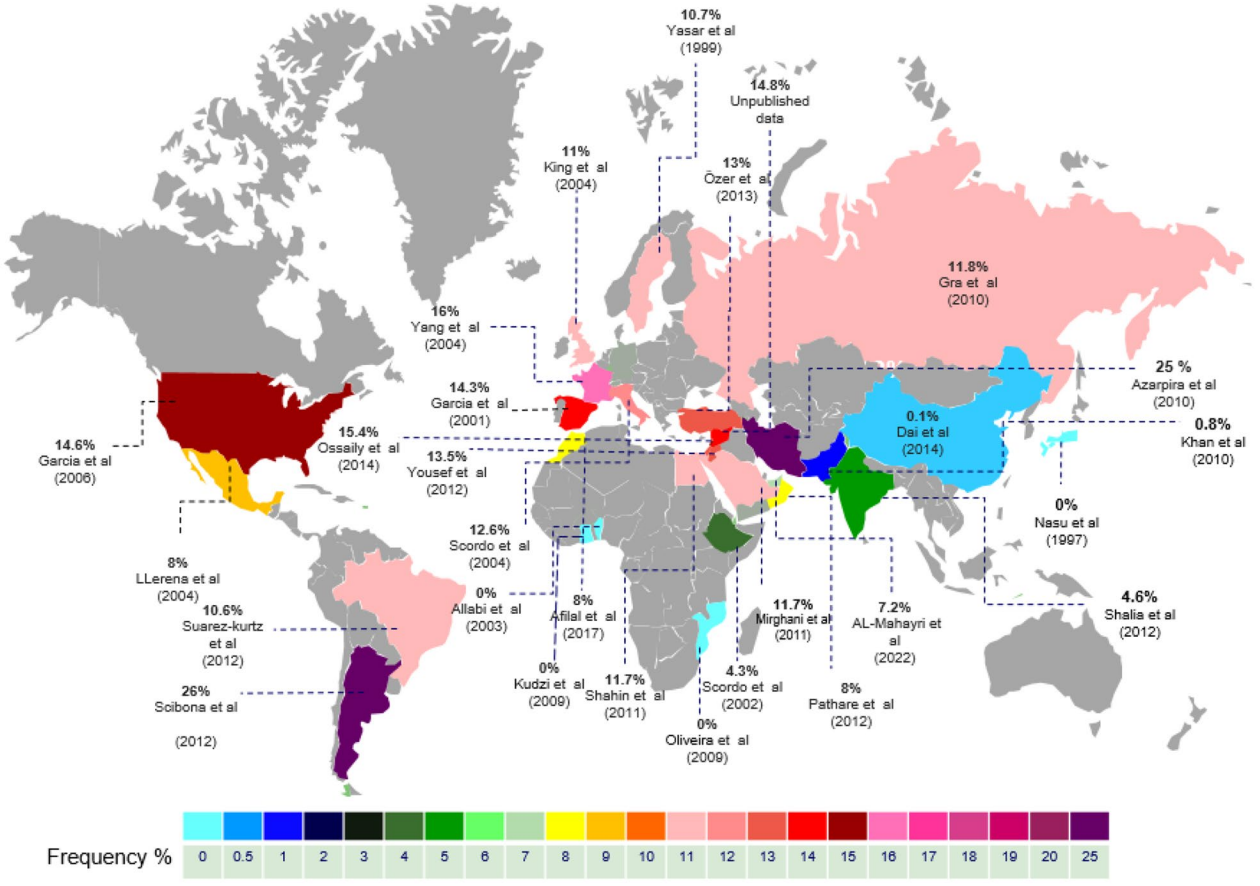

Fig. 2 Map of the frequency distribution of the *CYP2C9*2* allele in 28 countries worldwide

CYP2C9 polymorphisms in several European and North American populations [27]. Expectedly, our study revealed a similar frequency of *CYP2C9*3* in Syrians with the prevalence observed in neighboring populations, such as Lebanese (7.8%) and Jordanians (6.8%) [28, 30] as well as other dominantly Arab populations, for instance Saudis (9.2%) and Egyptians (9.1%) [34, 36]. The highest prevalence *CYP2C9*3* in the Arabian Gulf countries was reported in the UAE (21.3%) and the lowest in Sultanate of Oman (5%) [32–33]. When comparing the prevalence rate of *CYP3C9*3* in our study cohort with those in the non-Arab Middle-Eastern countries, we conclude similarity with that of Iranians (9.8%), but significantly lower frequency than that reported in Turks (15%), who proved to have the highest prevalence rate in the Middle-East ($p = 0.022$) [20, 31].

The highest prevalence of *CYP2C9*3* in European countries was reported in Spain (16.2%), nevertheless, the frequencies were much lower in Italy (9.7%), the United Kingdom (8.5%), France (8%), and Sweden (7.4%), and as low as 5% in Russia [37–42].

Although the *CYP2C9*3* allele is more common in East Asians than the **2* allele, the documented prevalence of *CYP2C9*3* was as low as 2.3% and 2.9% in Japan and China, respectively [16, 48]. To the contrary, an evidently higher prevalence is observed in South Asia, with frequencies of 7.5% and 12.2% in Pakistan and India, respectively [47, 53].

Moreover, similar to the almost absent **2* allele, the prevalence of the **3* allele in Africans is the lowest globally, as it does not exceed 3.2% and 1% in Ethiopia and Mozambique, respectively [45–46], and is almost zero (0%) in the sub-Saharan countries such as Benin and Ghana [43–44]. In South America, a low *CY2C29*3* prevalence is reported in Argentina (3%), but doubled in neighboring Brazil (6%), and significantly increased to the north; such as in Mexico (6.6%) and North America (7%) [49–52]. Figure 3 illustrates the *CY2C29*3* frequencies in Syrians, Middle Eastern countries and globally.

Our findings support a similar profile of *CYP2C9* polymorphisms distribution with that reported in several European populations, not only with the *CYP2C9*2* followed by the *CYP2C9*3* being the most abundant, but also in the absence of alleles such as **6*, **8*, **9*, and **11*, which are common among Africans [14]. The prevalence of *CYP2C9*8* and **11* in Mozambique is documented at 14.6% and 2.4%, respectively, however, the **11* allele frequency does not exceed 0.4% in some European countries

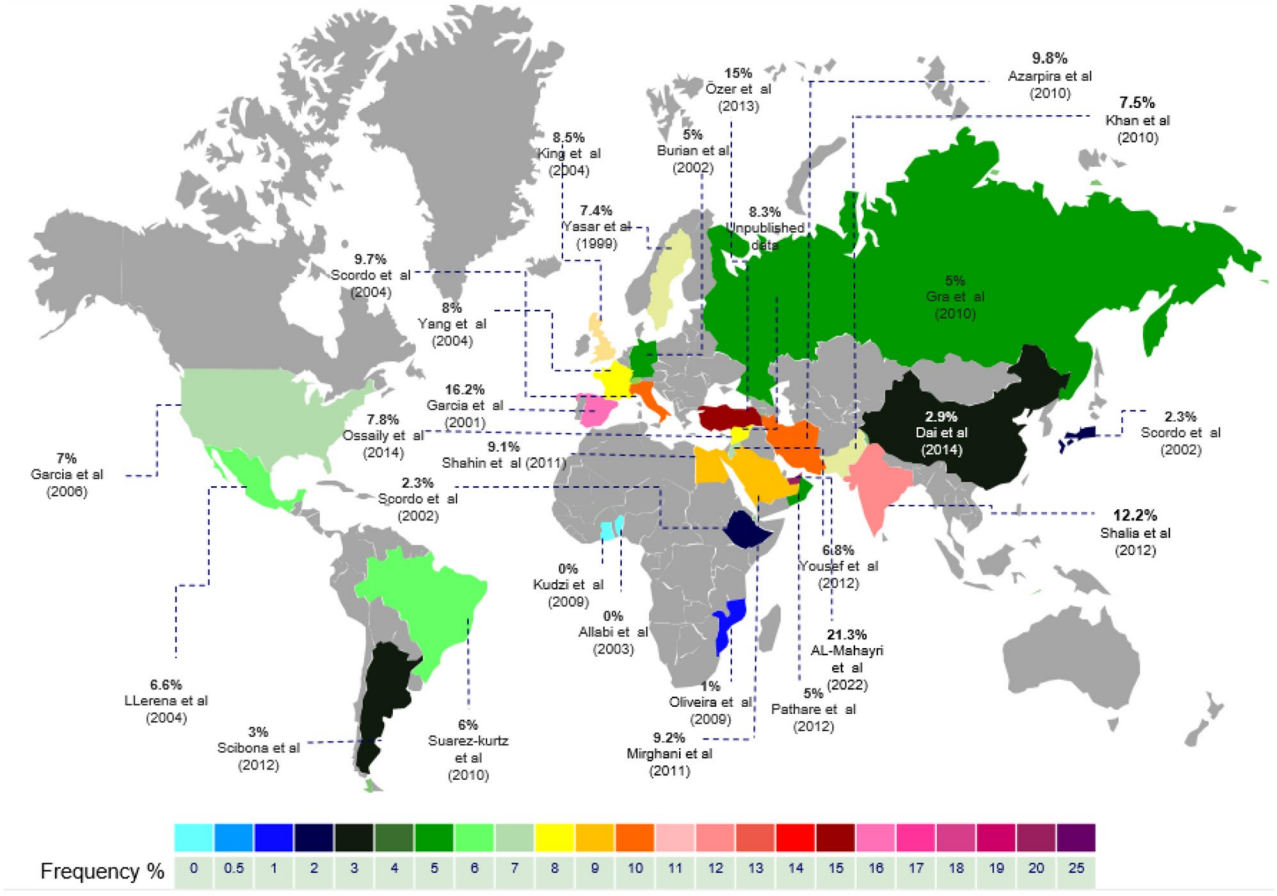

Fig. 3 Map of the frequency distribution of the *CYP2C9*3* allele in 28 countries worldwide

such as Belgium [43, 54]. Another aspect of similarity manifests in the scarcity of some alleles that were first discovered in Asians, but lack any evidence of their existence in European populations [11, 16]. Of the 138 subjects constituted our study cohort, three individuals carried one copy of the rare *CYP2C9*41* ($n = 2$, 0.72%) or **46* allele ($n = 1$, 0.36%). To the best of our knowledge, this is the first time the *CYP2C9*41* and **46* allele reported in non-Chinese populations and at higher frequencies compared with the frequencies documented in Chinese (0.047% and 0.024%, respectively) [16].

*CYP2C9*41* is characterized by a Lys > Arg exchange in exon 3, and *CYP2C9*46* is associated with Ala > Pro exchange in the same exon. In vitro studies that assessed the enzymatic efficacy of the two variants with Diclofenac as a substrate in comparison with the *CYP2C9*1/*1* as a control, have shown an attenuation by 70% in enzyme efficacy due to the *CYP2C9*46* allele, but no inferiority in the efficacy related to the *CYP2C9*41* allele compared to the wild type allele [16]. The only in vivo evidence of a reduced enzyme activity associated with *CYP2C9*46* was documented by Aldiban et al. in a case-report of a patient on warfarin with *the CYP2C9*1/*46* genotype who demanded a 62% reduction in the warfarin dosage for safe and effective treatment [55].

The comparisons we have set between our findings with other Middle Eastern countries emphasize homogeneity of *CYP2C9* allele frequencies in the Levant countries and populations, but considerable variation when compared with different Middle Eastern populations and ethnicities (e.g. Iranians and Turks).

The intronic SNV (rs9332120) investigated in this current study at frequencies of 12.3%, is unlikely to have any impact on the function or expression of the CYP2C9 enzyme [56]. In contrast, a close association between CYP2C9*2 (rs1799853) and rs9332119 in intron 1 was revealed. The frequency (6.1%) of the intronic rs9332119 allele in our cohort is less than that (11%) reported by Venestra et al. in 192 Caucasian subjects [57].

The significance of this linkage resides in resolving the controversy surrounding the interpretation of the low-dose requirements of warfarin for individuals carriage the rs9332119, which can be explained by their concomitant carrying of the rs1799853 (*CYP2C9*2*) allele, which is translated into a decreased or abolished enzyme function.

CYP2C9 functionality is crucial for dosing a variety of commonly prescribed as well as over the counter drugs. Based on our extrapolation of the functional consequences of the observed genotypes on the *CYP2C9* metabolic phenotypes, normal metabolizers (NMs) are the most prevalent phenotype in Syrians, accounting for 56.5% of the population, followed by intermediate metabolizer (IM) and poor metabolizer (PM) phenotypes that constitute 36.2% and 5% of the population, respectively. The prevalence reported in this study is in agreement with these observed in Europeans, other Middle Eastern populations and South Asians, but considerably diverges from that in East Asians, where more than 90% of the population are phenotypically categorized as normal metabolizers, with the remaining individuals classified as intermediate metabolizers [58], as elucidated in Fig. 4.

PMs and IMs are more prone to adverse effects and toxicity of narrow therapeutic drugs, such as warfarin-induced hemorrhages, glipizide-provoked hypoglycemia, phenytoin related neurotoxicity, severe skin rash and hepatotoxicity, and increased risk of gastrointestinal bleeding, hypertension and myocardial infarction upon treatment with non-steroidal anti-inflammatory drugs (NSAIDs) [59–61]. Our data indicate that up to 43.5% of Syrian patients might be at greater risk for higher drug exposure even though they are prescribed and receiving regular/standard doses of warfarin, phenytoin, glipizide, NSAIDs, and other *CYP2C9* drug substrates and therefore they might benefit from pharmacogenetics-guided dose reductions of these drugs.

On the contrary, individuals classified as IMs and PMs who receive treatment with pro-drugs, such as losartan, which undergoes CYP2C9-mediated oxidation to be converted into its active metabolite (carboxylic acid E-3174), may experience reduced clinical benefits [61].

Evidence-based guidelines with specific drug and genetic recommendations are crucial for applying pharmacogenetics knowledge in clinical practice. The Dutch Pharmacogenetics Working Group (DPWG), the Clinical Pharmacogenetics Implementation Consortium (CPIC), the FDA and European drug Agency (EMA) have been developing these guidelines for over a decade [62]. It is ideal to provide these recommendations to healthcare providers at the time of prescribing or dispensing a medication for a patient with *CYP2C9* genotype that necessitates an action, such as a dose adjustment. Here, we conduct a comparison between concerned organizations according to *CYP2C9* pharmacogenomics guidelines (Table 3).

We acknowledge that the cohort of our study is confined to two Syrian cities (Damascus and Homs) and that genetic information of small geographically defined groups cannot provide accurate estimations of national allele frequencies in highly diverse populations, such as Syrians. However, we believe that our cohort well represent the Syrian population, as Damascus is the political and economic capital of the country, and is the most diverse city in Syria with an admixture population from all around the different Syrian governorates. Moreover, over-decade crisis that caused massive displacement of

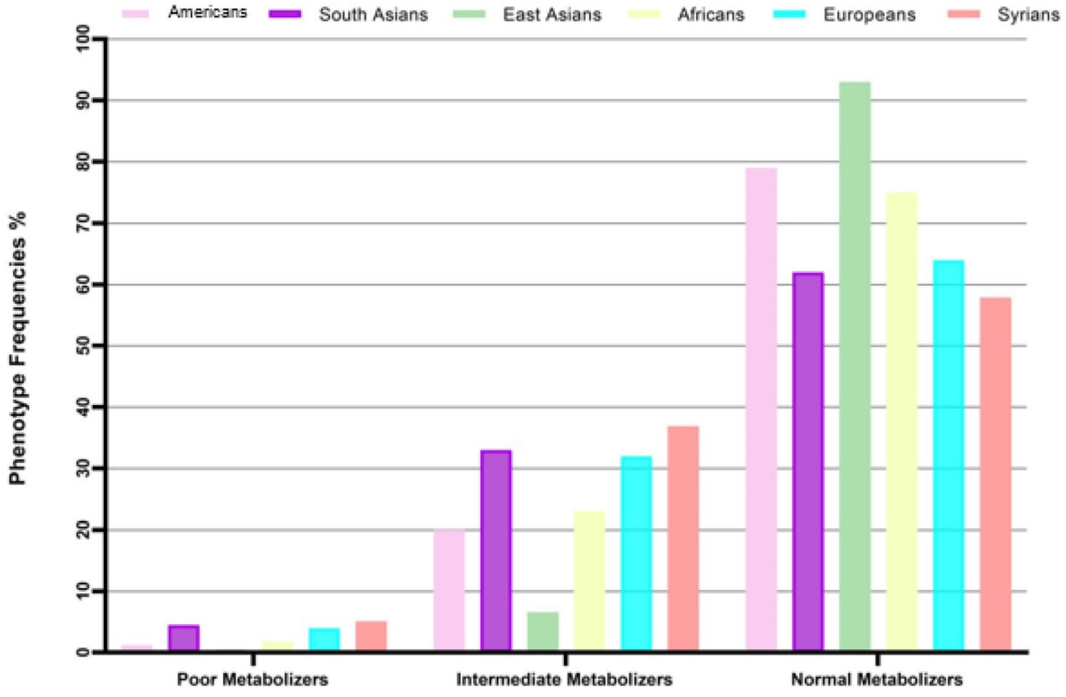

Fig. 4 The frequencies of CYP2C9 phenotypes in different populations

Table 3 CYP2C9 pharmacogenetics recommendations according to different drug regulatory agencies

Drug Name	Regulatory Agencies	Recommendation
Avatrombopag	CPIC	N.A
	DPWG	N.A
	EMA	Caution must also be exercised in patients with loss-of-function polymorphisms of *CYP2C9*, as these can increase avatrombopag exposure
	FDA	N.A
	FIDMD	N.A
	MEB	N.A
	HCSC	Caution must also be exercised in patients with loss-of-function polymorphisms of *CYP2C9*, as these can increase avatrombopag exposure
Celecoxib	CPIC	Use the lowest effective dosage for shortest duration consistent with individual patient treatment goals. Alternatively, consider an alternate therapy not metabolized by *CYP2C9* or not significantly impacted by *CYP2C9* genetic variants in vivo.
	DPWG	N.A
	EMA	Patients who are known or suspected to be *CYP2C9* poor metabolizers based on genotyping or previous history/experience with other *CYP2C9* substrates should be administered celecoxib with caution, as the risk of dose-dependent adverse effects is increased. Consider starting treatment at a reduced dose.
	FDA	Patients who are known or suspected to be poor *CYP2C9* metabolizers initiate treatment with half of the lowest recommended dose. In patients with juvenile rheumatoid arthritis who are known or suspected to be poor *CYP2C9* metabolizers, consider using alternative treatments.
	FIDMD	N.A
	MEB	N.A
	HCSC	Patients who are known, or suspected to be *CYP2C9* poor metabolizers based on previous history/experience with other *CYP2C9* substrates should be administered celecoxib with caution
Dronabinol	CPIC	N.A
	DPWG	N.A
	EMA	N.A
	FDA	Monitoring for increased adverse reactions is recommended in patients known to carry genetic variants associated with diminished *CYP2C9* function.
	FIDMD	N.A
	MEB	N.A
	HCSC	N.A
Erdafitinib	CPIC	N.A
	DPWG	N.A
	EMA	N.A.
	FDA	Monitor for increased adverse reactions in patients who are known or suspected to have *CYP2C9*3/*3* genotype.
	FIDMD	N.A
	MEB	N.A
	HCSC	Patients known to have this genotype should be monitored for increased adverse reactions
Flurbiprofen	CPIC	N.A
	DPWG	N.A
	EMA	N.A.
	FDA	Reduce the dose of flurbiprofen in patients who are known or suspected to be poor *CYP2C9* metabolizers based on genotype or previous history/experience with other *CYP2C9* substrates.
	FIDMD	N.A
	MEB	N.A
	HCSC	N.A
Lesinurad	CPIC	N.A
	DPWG	N.A
	EMA	Patients known to be poor metabolizers should be treated with caution, as the potential risk of renal-related adverse effects may be increased
	FDA	Recommendation: use with caution in *CYP2C9* poor metabolizer.
	FIDMD	N.A
	MEB	N.A
	HCSC	N.A

Table 3 (continued)

Drug Name	Regulatory Agencies	Recommendation
Meloxicam	CPIC	The recommendations are to either initiate therapy with 50% of the lowest recommended starting dose or choose an alternative therapy, consistent with the recommendations in PMs for short half-life NSAIDs
	DPWG	N.A
	EMA	N.A
	FDA	Consider dose reductions in poor metabolizers. Monitor patients for adverse reactions.
	FIDMD	N.A
	MEB	N.A
	HCSC	N.A
Phenytoin	CPIC	Consider dose adjustment
	DPWG	Consider dose adjustment
	EMA	N.A
	FDA	Pharmacogenomic information
	FIDMD	Dose reduction along with monitoring of plasma concentrations may be necessary.
	MEB	Pharmacogenomic information.
	HCSC	N.A
Piroxicam	CPIC	N.A
	DPWG	N.A
	EMA	N.A
	FDA	Consider dose reduction in patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history with other CYP2C9 substrates.
	FIDMD	N.A
	MEB	N.A
	HCSC	N.A
Warfarin	CPIC	Calculate dose based on validated published pharmacogenetic algorithm.
	DPWG	They recommend reducing warfarin dose for CYP2C9 poor and intermediate metabolizers (PM and IM) and patients with *CYP2C9*1/*3*, **2/*3*, **2/*2* or *3/*3 genotype. The genotype-specific initial dose and maintenance dose can be calculated using an algorithm
	EMA	N.A
	FDA	Strong recommendation: specific dose adjustment.
	FIDMD	N.A
	MEB	N.A
	HCSC	Patients carrying one or two copies of the *CYP2C9*2 or *3* alleles may require a decreased mean daily warfarin dose,
Natiglinide	FDA	Dosage reduction is recommended for poor metabolizers. Increase monitoring frequency for adverse reactions
Siponimod	CPIC	N.A
	DPWG	In patients with a CYP2C9*3*3 genotype, avoid siponimod
	EMA	In patients with a CYP2C9*3*3 genotype, siponimod should not be used
	FDA	Contraindicated in patients who have a *CYP2C9*3/*3* genotype
	FIDMD	N.A
	MEB	N.A
	HCSC	Contraindicated in patients with a *CYP2C9*3/*3* genotype. Dose adjustments are recommended for patients with *CYP2C9*1/*3* or a *CYP2C9*2/*3* genotype.

CPIC: Clinical Pharmacogenetics Implementation Consortium; **FDA**: Food and Drug Administration; **EMA**: European Medicines Agency; **DPWG**: Dutch Pharmacogenetics Working Group; **FIDMD**: Federal Institute for Drugs and Medical Devices; **MEB**: Medicines Evaluation Board; **HCSC**: Health Canada (Santé Canada)

the Syrian population has turned the capital Damascus into a relatively "safer zone" for tens of thousands of displaced Syrians, and therefore increased the diversity of the residents. Homs, on the other hand, is located in the center of the country; with a population representing the central region as well as significant descendants of inhabitants of theSyrian Mediterranean coast. The comparable frequencies of the investigated alleles, genotypes, and phenotypes between the subpopulations of the two cities support our claims of the similar genetic makeup and homogeneity within study population.

The advent of advanced sequencing methods has led to a revolutionary surge in the detection of variants of uncertain significance (VUS). As such, further research is required to unravel the roles and prevalence of VUS to classify them effectively.

Conclusions

This cross-sectional study is the first conducted in Syria to explore 26 SNVs in the *CYP2C9* encoding gene. Our findings reveal high abundance of *CYP2C9*2* followed by *CYP2C9*3* SNVs, the two most prominent functionally abolished alleles in European and Middle Eastern populations. Moreover, our findings prove absence of other functionally reduced activity *CYP2C9* alleles (*CYP2C9*5* and **8*) previously identified at relatively high prevalence in populations with African ancestries. Additionally, this study identified two novel SNVs (**41* and **46*) that have not been previously reported outside East Asian populations.

Our findings highlight the importance of conducting further *CYP2C9* genotyping studies among Syrians and other Middle Eastern populations in order to gain insights into their genetic makeup. This study could ultimately contribute to the development/adaptation of personalized algorithms based on individuals' genetic profiles in this part of the world, thereby assisting healthcare providers in making informed decisions regarding the prescription and dosing of medications with a narrow therapeutic index.

Abbreviations

BP	Base Pair
CYP2C9	Cytochrome P450 2C9
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamin Tetraacetic Acid
FDA	Food and Drug Administration
IMs	Intermediate Metabolizers
LD	Linkage Disequilibrium
PCR	Polymerase Chain Reaction
PMs	Poor Metabolizers
SNVs	Single nucleotide variants
VUS	Variants of uncertain significance

Acknowledgements

Not applicable.

Author contributions

All authors had read and approved the final version of the manuscript. WA was responsible for data acquisition, laboratory work, statistical analyses, data interpretation, and drafting the manuscript. MNA co-supervised the project and revised the manuscript. LAY conceived and designed the study, co-supervised the project and was involved in data interpretation, co-writing, and final revision of the manuscript.

Funding

This work was supported by a special fund provided by the University of Damascus and The National Commission for Biotechnology-Damascus.

Data availability

The datasets generated and analyzed during the current study are available in the ClinVar repository, with the accession numbers SCV005397924, SCV005397925, SCV005397926 and SCV005397927.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Scientific Research Ethics Committee at the Faculty of Pharmacy, Damascus University (Number:1,issued on April 25th, 2016). All individuals gave their signed informed consents.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 18 October 2024 / Accepted: 29 January 2025
Published online: 13 February 2025

References

1. Céspedes-garro C, Fricke-galindo I, Naranjo MEG, Rodrigues-soares F, Fariñas H, Andrés F, De et al. Worldwide interethnic variability and geographical distribution of CYP2C9 genotypes and phenotypes. Expert Opinion on Drug Metabolism and Toxicology. 2015;5255.
2. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol. 1998;45(6):525–38. https://doi.org/10.1046/j.1365-2125.1998.00721.x. PMID: 9663807; PMCID: PMC1873650.
3. Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress. Fertil Steril. 2018;109(6):952–63. https://doi.org/10.1016/j.fertnstert.2018.05.006. PMID: 29935653; PMCID: PMC6366451.
4. Wijnen PA, Op den Buijsch RA, Drent M, Kuijpers PM, Neef C, Bast A, Bekers O, Koek GH. Review article: the prevalence and clinical relevance of cytochrome P450 polymorphisms. Aliment Pharmacol Ther. 2007;26(Suppl 2):211–9. https://doi.org/10.1111/j.1365-2036.2007.03490.
5. Sangkuhl K, et al. PharmVar GeneFocus: CYP2C9. Clin Pharmacol Ther. 2021;110(3):662–76. https://doi.org/10.1002/cpt.2333. Epub 2021 Jul 12. PMID: 34109627; PMCID: PMC8607432.
6. Ding Y, Yang D, Zhou L, He P, Yao J, Xie P, Lin D, Sun D, Sun P, Li Q, Geng T, Jin T. Cytochrome P450 2C9 (CYP2C9) polymorphisms in Chinese Li population. Int J Clin Exp Med. 2015;15(11):21024–33. PMID: 26885033; PMCID: PMC4723878.
7. Daly AK, Rettie AE, Fowler DM, Miners JO. Pharmacogenomics of CYP2C9: Functional and Clinical Considerations. J Pers Med. 2017. 28;8(1):1. https://doi.org/10.3390/jpm8010001. PMID: 29283396; PMCID: PMC5872075.
8. Schwarz UI. Clinical relevance of genetic polymorphisms in the human CYP2C9 gene. Eur J Clin Invest. 2003;33 Suppl 2:23–30. https://doi.org/10.1046/j.1365-2362.33.s2.6.x. PMID: 14641553.
9. Maekawa K, Harakawa N, Sugiyama E, Tohkin M, Kim SR, Kaniwa N, Katori N, Hasegawa R, Yasuda K, Kamide K, Miyata T, Saito Y, Sawada J. Substrate-dependent functional alterations of seven CYP2C9 variants found in Japanese subjects. Drug Metab Dispos. 2009;37(9):1895–903. https://doi.org/10.1124/dmd.109.027003. Epub 2009 Jun 18. PMID: 19541829.
10. Holm J, Lindh JD, Andersson ML, Mannheimer B. The effect of amiodarone on warfarin anticoagulation: a register-based nationwide cohort study involving the Swedish population. J Thromb Haemost. 2017;15(3):446–53. https://doi.org/10.1111/jth.13614. Epub 2017 Feb 17. PMID: 28058824.
11. Zhao F, Loke C, Rankin SC, Guo JY, Lee HS, Wu TS, Tan T, Liu TC, Lu WL, Lim YT, Zhang Q, Goh BC, Lee SC. Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. Clin Pharmacol Ther. 2004;76(3):210-9. https://doi.org/10.1016/j.clpt.2004.05.005. PMID: 15371982.
12. Dean L, Warfarin Therapy. and VKORC1 and CYP Genotype. 2012 [Updated 2018 Jun 11]. In: Pratt VM, Scott SA, Pirmohamed M, editors. Medical Genetics Summaries [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2012-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK84174/
13. Waounou M, et al. The impact of CYP2C9*11 alllic. Clin Pharmacol Thraputics. 2022;1121:156–63.
14. Pratt VM, Cavallari LH, Tredici L, Del, Hachad H, Ji Y, Moyer AM et al. Recommendations for Clinical CYP2C9 Genotyping Allele Selection A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagnostics [Internet]. 2019;21(5):746–55. Available from: https://doi.org/10.1016/j.jmoldx.2019.04.003
15. Kramer MA, Rettie AE, Rieder MJ, Cabacungan ET, Hines RN. Novel CYP2C9 promoter variants and assessment of their impact on gene expression. Mol Pharmacol. 2008;73(6):1751–60. https://doi.org/10.1124/mol.107.044149. Epub 2008 Feb 29. PMID: 18310303; PMCID: PMC2413059.
16. Dai DP, et al. CYP2C9 polymorphism analysis in Han Chinese populations: building the largest allele frequency database. Pharmacogenomics J. 2014;14(1):85–92. https://doi.org/10.1038/tpj.2013.2. Epub 2013 Feb 12. PMID: 23400009.

17. Kim JA, Ceccarelli R, Lu CY. Pharmacogenomic Biomarkers in US FDA-Approved Drug Labels (2000–2020). 2021.
18. Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. Pharmacogenetics. 2002;12(3):251–63.
19. Al-saikhan FI. Saudi Journal of Biological Sciences Genetic risk assessment towards warfarin application: Saudi Arabia study with a potential to predict and prevent side effects. Saudi J Biol Sci [Internet]. 2020;27(1):456–9. Available from: https://doi.org/10.1016/j.sjbs.2019.11.007
20. Azarpira N, Namazi S, Hendijani F, Banan M, Darai M. Investigation of allele and genotype frequencies of CYP2C9, CYP2C19 and VKORC1 in Iran. Pharmacol Reports [Internet]. 2010;62(4):740–6. Available from: https://doi.org/10.1016/S1734-1140(10)70332-7
21. Ahmed S, Altaf N, Ejaz M, Altaf A, Amin A. Variations in the frequencies of polymorphisms in the CYP2C9 gene in six major ethnicities of Pakistan. Sci Rep [Internet]. 2020;1–8. Available from: https://doi.org/10.1038/s41598-020-76366-x
22. Marjani A, Mohammad A. Genetic polymorphism of CYP2C9 among Sistani Ethnic Group in Gorgan. Indian J Clin Biochem. 2018;33(2):208–13.
23. Bader LA, Elewa H. The impact of genetic and non-genetic factors on Warfarin Dose Prediction in MENA Region. Syst Rev. 2016;11(12):1–15.
24. Fekete F, Mangó K, Déri M, et al. Impact of genetic and non-genetic factors on hepatic CYP2C9 expression and activity in Hungarian subjects. Sci Rep. 2021;17081. https://doi.org/10.1038/s41598-021-96590-3.
25. Maekawa K, Fukushima-Uesaka H, Tohkin M, Hasegawa R, Kajio H, Kuzuya N, Yasuda K, Kawamoto M, Kamatani N, Suzuki K, Yanagawa T, Saito Y, Sawada J. Four novel defective alleles and comprehensive haplotype analysis of CYP2C9 in Japanese. Pharmacogenet Genomics. 2006;16(7):497–514. https://doi.org/10.1097/01.fpc.0000215069.14095.c6. PMID: 16788382.
26. Amorosi CJ, Chiasson MA, Mcdonald MG, Wong LH, Sitko KA, Boyle G et al. Massively parallel characterization of CYP2C9 variant enzyme activity and abundance. Am J Hum Genet [Internet]. 2021;108(9):1735–51. Available from: https://doi.org/10.1016/j.ajhg.2021.07.001
27. Mak M, Lam C, Pineda SJ, Lou M, Xu LY, Meeks C, Lin C, Stone R, Rodgers K, Mitani G. Pharmacogenetics of Warfarin in a Diverse Patient Population. J Cardiovasc Pharmacol Ther. 2019;24(6):521–33. Epub 2019 May 7. PMID: 31064211.
28. Ossaily S, Zgheib NK. The pharmacogenetics of drug metabolizing enzymes in the Lebanese population. Pharmacogenomic Stud. 2014;29(2):81–90.
29. Ayesh BM, Abu Shaaban AS, Abed AA. Evaluation of CYP2C9- and VKORC1-based pharmacogenetic algorithm for warfarin dose in Gaza-Palestine. Future Sci OA. 2018 10;4(3):FSO276. https://doi.org/10.4155/fsoa-2017-0112. PMID: 29568565; PMCID: PMC5859345.
30. Yousef AM, Bulatova NR, Newman W, Hakooz N, Ismail S, Qusa H, Zahran F, Anwar Ababneh N, Hasan F, Zaloom I, Khayat G, Al-Zmili R, Naffa R, Al-Diab O. Allele and genotype frequencies of the polymorphic cytochrome P450 genes (CYP1A1, CYP3A4, CYP3A5, CYP2C9 and CYP2C19) in the Jordanian population. Mol Biol Rep. 2012;39(10):9423–33. https://doi.org/10.1007/s11033-012-1807-5. Epub 2012 Jun 22. PMID: 22722998.
31. Özer M, Demirci Y, Hizel C, Sarikaya S, Karalti İ, Kaspar Ç, Alpan S, Genç E. Impact of genetic factors (CYP2C9, VKORC1 and CYP4F2) on warfarin dose requirement in the Turkish population. Basic Clin Pharmacol Toxicol. 2013;112(3):209–14. https://doi.org/10.1111/bcpt.12024. Epub 2012 Dec 6. PMID: 23061746.
32. Al-Mahayri ZN, Khasawneh LQ, Alqasrawi MN, Altoum SM, Jamil G, Badawi S, Hamza D, George L, AlZaabi A, Ouda H, Al-Maskari F, AlKaabi J, Patrinos GP, Ali BR. Pharmacogenomics implementation in cardiovascular disease in a highly diverse population: initial findings and lessons learned from a pilot study in United Arab Emirates. Hum Genomics. 2022;25(1):42. https://doi.org/10.1186/s40246-022-00417-9. PMID: 36154845; PMCID: PMC9509637.
33. Mirghani RA, Chowdhary G, Elghazali G. Distribution of the Major Cytochrome P450 (CYP) 2C9 Genetic Variants in a Saudi Population. Basic & clinical pharmacology & toxicology. 2011;450:111–4.
34. Pathare AV, Al Zadjali S, Misquith R, Alkindi SS, Panjwani V, Lapoumeroulie C, Pravin S, Paldi A, Krishnamoorthy R. Warfarin pharmacogenetics: polymorphisms of the CYP2C9, CYP4F2, and VKORC1 loci in a genetically admixed Omani population. Hum Biol. 2012; 84(1):67–77. https://doi.org/10.3378/027.084.0103. PMID: 22452429.
35. Afilal D, Basselam MA, Brakez Z, Chouham S, Brehm A, Izaabel EH. Genetic polymorphism of drug-metabolizing enzymes CYP2C9 and CYP2C19 in Moroccan Population. Genet Test Mol Biomarkers. 2017;21(5):298–304. https://doi.org/10.1089/gtmb.2016.0304. Epub 2017 Mar 10. PMID: 28282224.
36. Shahin MH, Khalifa SI, Gong Y, Hammad LN, Sallam MT, El Shafey M, Ali SS, Mohamed ME, Langaee T, Johnson JA. Genetic and nongenetic factors associated with warfarin dose requirements in Egyptian patients. Pharmacogenet Genomics. 2011;21(3):130–5. https://doi.org/10.1097/FPC.0b013e3283436b86. PMID: 21228733; PMCID: PMC3038469.
37. Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E. Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. Pharmacol Res. 2004;50(2):195–200. https://doi.org/10.1016/j.phrs.2004.01.004. PMID: 15177309.
38. García-Martín E, Martínez C, Ladero JM, Gamito FJ, Agúndez JA. High frequency of mutations related to impaired CYP2C9 metabolism in a Caucasian population. Eur J Clin Pharmacol. 2001;57(1):47– 9. https://doi.org/10.1007/s002280100264. PMID: 11372590.
39. Yang JQ, Morin S, Verstuyft C, Fan LA, Zhang Y, Xu CD, Barbu V, Funck-Brentano C, Jaillon P, Becquemont L. Frequency of cytochrome P450 2C9 allelic variants in the Chinese and French populations. Fundam Clin Pharmacol. 2003;17(3):373-6. https://doi.org/10.1046/j.1472-8206.2003.00148.x. PMID: 12803577.
40. Gra O, Mityaeva O, Berdichevets I, Kozhekbaeva Z, Fesenko D, Kurbatova O, Goldenkova-Pavlova I, Nasedkina T. Microarray-based detection of CYP1A1, CYP2C9, CYP2C19, CYP2D6, GSTT1, GSTM1, MTHFR, MTRR, NQO1, NAT2, HLA-DQA1, and AB0 allele frequencies in native Russians. Genet Test Mol Biomarkers. 2010;14(3):329–42. https://doi.org/10.1089/gtmb.2009.0158. PMID: 20373852.
41. King BP, Khan TI, Aithal GP, Kamali F, Daly AK. Upstream and coding region CYP2C9 polymorphisms: correlation with warfarin dose and metabolism. Pharmacogenetics. 2004;14(12):813– 22. https://doi.org/10.1097/00008571-200412000-00004. PMID: 15608560.
42. Yasar U, Eliasson E, Dahl ML, Johansson I, Ingelman-Sundberg M, Sjöqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. Biochem Biophys Res Commun. 1999;254(3):628– 31. https://doi.org/10.1006/bbrc.1998.9992. Erratum in: Biochem Biophys Res Commun 1999;258(1):227. PMID: 9920790.
43. Allabi AC, Gala JL, Desager JP, Heusterspreute M, Horsmans Y. Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations. Br J Clin Pharmacol. 2003;56(6):653–7. https://doi.org/10.1046/j.1365-2125.2003.01937.x. PMID: 14616425; PMCID: PMC1884305.
44. Kudzi W, Dodoo AN, Mills JJ. Characterisation of CYP2C8, CYP2C9 and CYP2C19 polymorphisms in a Ghanaian population. BMC Med Genet. 2009;10:124. https://doi.org/10.1186/1471-2350-10-124. PMID: 19954515; PMCID: PMC3224726.
45. Oliveira E, Pereira R, Amorim António, McLeod, Howard, Prata M. Patterns of Pharmacogenetic Diversity in African populations: role of ancient and recent history. Pharmacogenomics. 2009;10:1413–22. https://doi.org/10.2217/pgs.09.74.
46. Scordo MG, et al. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Pharmacogenet Genomics. 2002;702–10. https://doi.org/10.1067/mcp.2002.129321.
47. Shalia KK, et al. Prevalence of VKORC1 and CYP2C9 gene polymorphisms in Indian population and its effect on warfarin response. J Assoc Physicians India. 2012;60:34–8.
48. Nasu K. Genetic analysis of CYP2C9 polymorphism in Japanese Population. Pharmacogenetics. 1997,7(5):405–9.
49. Scibona P, Redal MA, Garfi LG et al. Prevalence of CYP2C9 and VKORC1 alleles in the Argentine population and implications for prescribing dosages of anticoagulants. Genetics and Molecular Research: GMR. 2012;11(1):70–76. https://doi.org/10.4238/2012.january.9.8. PMID: 22290467.
50. Suarez-Kurtz G, Pena SD, Struchiner CJ, Hutz MH. Pharmacogenomic diversity among brazilians: influence of Ancestry, Self-reported Color, and geographical origin. Front Pharmacol. 2012;6:3:191. https://doi.org/10.3389/fphar.2012.00191. PMID: 23133420; PMCID: PMC3490152.
51. LLerena A, Dorado P, O'Kirwan F, Jepson R, Licinio J, Wong ML. Lower frequency of CYP2C9*2 in Mexican-Americans compared to Spaniards. Pharmacogenomics J. 2004;4(6):403-6. https://doi.org/10.1038/sj.tpj.6500278. PMID: 15452553.
52. Zhou Y, Nevosadová L, Eliasson E, Lauschke VM. Global distribution of functionally important CYP2C9 alleles and their inferred metabolic consequences. Hum Genomics. 2023; 28;17(1):15. https://doi.org/10.1186/s40246-023-00461-z. PMID: 36855170; PMCID: PMC9976394.
53. Khan D, Khan F. Impact of CYP2C9 genetic polymorphism on warfarin dose requirements in Pakistani population. Pak J Pharm Sci. 2010;23:417–22.

54. Wanounou M, Shaul C, Abu Ghosh Z, Alamia S, Caraco Y. The impact of CYP2C9*11 allelic variant on the pharmacokinetics of Phenytoin and (S)-Warfarin. Clin Pharmacol Ther. 2022;112(1):156–63. Epub 2022. PMID: 35426132; PMCID: PMC9322346.
55. Aldiban W, Altawil Y, Hussein S, et al. Hyper-responsiveness to warfarin in a young patient with the VKORC1 -1639GA/CYP2C9*1*46 genotype: a case report. Thromb J. 2022;20:65. https://doi.org/10.1186/s12959-022-00425-8.
56. Di D, Ciccacci C, Ru S, Forte V, Novelli G, Borgiani P. Resequencing of VKORC1, CYP2C9 and CYP4F2 genes in Italian patients requiring extreme low and high warfarin doses. Thromb Res. 2013;132:123–6.
57. Veenstra DL, Blough DK, Higashi MK, Farin FM, Srinouanprachan S, Rieder MJ, Rettie AE. CYP2C9 haplotype structure in European American warfarin patients and association with clinical outcomes. Clin Pharmacol Ther. 2005;77(5):353–64. https://doi.org/10.1016/j.clpt.2005.01.019. PMID: 15900281.
58. Tornio A, Backman JT. Cytochrome P450 in Pharmacogenetics: An Update. Adv Pharmacol. 2018;83:3–32. https://doi.org/10.1016/bs.apha.2018.04.007. PMID: 29801580.
59. Fohner AE, Rettie AE, Thai KK, Ranatunga DK, Lawson BL, Liu VX, Schaefer CA. Associations of CYP2C9 and CYP2C19 pharmacogenetic variation with Phenytoin-Induced cutaneous adverse drug reactions. Clin Transl Sci. 2020;13(5):1004–9. https://doi.org/10.1111/cts.12787. Epub 2020;18. PMID: 32216088; PMCID: PMC7485959.
60. Aquilante CL, Lam YWF. Chapter 7– The Role of Pharmacogenomics in Diabetes, HIV Infection, and Pain Management [Internet]. Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation. Elsevier; 2013. 247–271 p. Available from: https://doi.org/10.1016/B978-0-12-391918-2.00007-X
61. Lam YWF, Cavallari LH. Principles of Pharmacogenomics: [Internet]. Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation. Elsevier; 2013. 1–44 p. Available from: https://doi.org/10.1016/B978-0-12-391918-2.00001-9
62. Abdullah-Koolmees H, van Keulen AM, Nijenhuis M, Deneer VHM. Pharmacogenetics guidelines: overview and comparison of the DPWG, CPIC, CPNDS, and RNPGx guidelines. Front Pharmacol. 2021;25:11:595219. https://doi.org/10.3389/fphar.2020.595219. PMID: 33568995; PMCID: PMC7868558.

Publisher's note