BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2019, VOL. 76, NO. 3, 150–152
https://doi.org/10.1080/09674845.2019.1595272

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BIOMEDICAL SCIENCE IN BRIEF

ABCB1 2677G>T single nucleotide polymorphism influences warfarin dose requirement for warfarin maintenance therapy

Gopisankar MG[a], Hemachandren M[b] and Surendiran A[a]

[a]Department of Pharmacology, JIPMER, Puducherry, India; [b]Department of CTVS, JIPMER, Puducherry, India

ARTICLE HISTORY Received 22 January 2019; Accepted 4 February 2019
KEYWORDS Warfarin; pharmacogenomics; pharmacogenetics

Warfarin, a commonly prescribed anticoagulant, minimises development and recurrence of thromboembolic episodes in conditions such as deep vein thrombosis, pulmonary embolism, cardiac valve replacement and atrial fibrillation. It is known to cause various adverse effects amongst which increased risk of bleeding is the most common and disabling adverse effect. The dose requirement of warfarin varies more than tenfold among patients [1]. Several factors involved in this are genetic and non-genetic. Non-genetic factors are mainly related to compliance. Other factors are age, diet, body weight, diagnosis and clinical status of the patient which may explain 17–20% of the overall variation in maintenance dose. Genotypes such as CYP2C9, VKORC1, CYP4F2, GGCX, etc. have been studied extensively and have a role in determining warfarin dose requirement. Models developed from these studies could explain around 50% of the variability of drug response. There are differences between Caucasians, African Americans, Asians and other ethnic populations in terms of genetic polymorphisms of drug metabolizing enzymes and other genes, thus population-specific algorithms need to be developed incorporating relevant genotypes [2,3].

ABCB1 gene (Adenosine Triphosphate Binding Cassette Gene) coding for P-glycoprotein (P-gp) is said to determine the dose of warfarin [1]. Altered function of P-gp due to polymorphisms will change the blood level of drugs causing adverse effects or reduced efficacy of drugs. Patients with *ABCB1 3435 TT* genotypes require higher dose of warfarin compared to CT and CC types [4], and there is strong evidence that the effect of *ABCB1 3435C>T* is due to linkage disequilibrium with the non-synonymous *ABCB1 2677G>T* polymorphism and thus the effect is due haplotype rather than single nucleotide polymorphism (SNP) alone [5–10]. Thus *ABCB1 2677G>T* is postulated to be an important determinant of warfarin dosage. Furthermore, the *ABCB1 1236T/2677T/3435T* haplotype is the most common haplotype in Indian populations (31–49%) raising the relevance of *ABCB1 2677G>T* SNP in our population [7]. As several studies have focussed on the C3435T SNP in ABCB1, and there are no reports regarding 2677G>T, we hypothesized a role for the latter in warfarin maintenance dose requirement.

We tested our hypothesis in patients attending the cardiothoracic and vascular surgery department of a tertiary care health centre. Institute Ethics Committee approval was obtained prior to study initiation. Written informed consent was obtained from each participant prior to recruitment, who had undergone cardiac valve replacement and was on warfarin maintenance therapy with a stable International Normalized Ratio (INR) range of 2–3 for three consecutive visits. Exclusion criteria were age <18 or >65 years, or comorbid conditions affecting the coagulation pathway, liver or renal disorder. Five ml of venous blood sample was collected along with details of warfarin therapy and INR recordings for the last three consecutive visits. The cellular fraction of the blood sample after centrifugation was used for DNA extraction by the standard phenol chloroform method. Genotyping of DNA for *ABCB1 2677G>T* was done by using quantitative real-time PCR. The genotype frequencies were tested for Hardy Weinberg equilibrium using Chi-square test. The median daily dose between the three genotype groups was statistically compared using Kruskal–Wallis Test. In dominant model of analysis, the dose requirement was compared using Mann–Whitney *U* test. Statistical analysis was performed using GraphPad Prism version 5.02 and $P < 0.05$ was considered as statistically significant.

A total of 210 patients fulfilling inclusion and exclusion criteria were recruited. The baseline characteristics of the patients were found to be similar between the genotype groups (Table 1). The observations of genotype frequencies followed Hardy Weinberg equilibrium. In the co-dominant model of analysis, a significant difference in dose requirement was observed between the genotype groups. *Post-hoc* pair-wise analysis identified a significant difference in dose requirement between GG versus GT and GG versus TT, with higher dose requirement among patients with GT or TT genotypes compared to normal genotype. There was no significant difference between GT versus TT (Table 2). In the dominant model there was

CONTACT Surendiran A surenmbbs@gmail.com Department of Pharmacology, JIPMER, Puducherry 605006, India

Table 1. Comparison of patient characteristics across *ABCB1 2677G>T* genotypes (*N* = 210).

Parameters	GG (*N* = 71)	GT (*N* = 91)	TT (*N* = 48)	All subjects
Age (years)	38.4 (11)	39.8 (11)	37.2 (11)	39 (11)
Gender *n* (%)				
Male	36 (50.7)	36 (39.6)	15 (68.8)	87 (41.4)
Female	35 (49.0)	55 (60.4)	33 (31.2)	123 (58.6)
Weight (Kg)	55.7 (9.2)	59.3 (10.9)	57.7 (12)	57.7 (10.7)
Height (cm)	160.8 (7.8)	162.2 (13)	160.8 (7.4)	161.5 (12)
BMI (kg/m^2)	21.5 (3.3)	22 (2.9)	22.1 (3.9)	22.5 (9.3)
Duration of therapy (months)	12 (6–168)	12 (6–96)	24 (8–84)	12 (6–120)
Duration of current dose (months)	3 (3–8)	3 (3–10)	3 (3–7)	3 (3–8)
Valve replaced, *n* (%)				
Mitral valve	52 (73.2)	64 (70.3)	36 (75)	152 (72.4)
Tricuspid valve	2 (2.8)	0 (0)	0 (0)	2 (1)
Aortic valve	9 (12.7)	17 (18.7)	9 (18.8)	35 (16.7)
Aortic and mitral valve	10 (14.1)	10 (11)	3 (6.3)	23 (11)
Major concomitant drugs, *n* (%)				
Phenoxymethyl penicillin	54 (76.1)	63 (69.2)	31 (64.6)	148 (70.5)
Furosemide	54 (76.1)	53 (58.2)	29 (60.4)	136 (64.8)
Digoxin	39 (54.9)	40 (44.0)	24 (50)	103 (49)
Potassium chloride	26 (36.6)	26 (28.6)	14 (29.2)	66 (31.4)
Atenolol	18 (25.4)	19 (20.9)	9 (18.8)	46 (21.9)
Aspirin	14 (19.7)	18 (19.8)	5 (10.4)	37 (17.6)
Spironolactone	12 (16.9)	11 (12.1)	10 (20.8)	33 (15.7)
Omeprazole	9 (12.7)	12 (13.2)	6 (12.5)	27 (12.9)
Enalapril	8 (11.3)	8 (8.8)	4 (8.3)	20 (9.5)
Metoprolol	2 (2.8)	12 (13.2)	6 (12.5)	20 (9.5)
Vitamin B complex	6 (8.5)	6 (6.6)	0 (0)	12 (5.7)
Famotidine	1 (1.4)	7 (7.7)	0 (0)	8 (3.8)
Thyroxine	3 (4.2)	0 (0)	2 (4.2)	5 (2.4)
Metformin	1 (1.4)	4 (4.4)	0 (0)	5 (2.4)

Data presented as mean(SD), n(%), or median(IQR).

a significant difference in maintenance dose between patients who had a normal genotype and patients with at least one variant allele. The dose requirement was higher among patients with variant allele (Table 2). Effect of other independent variables in daily dose of warfarin was analysed by multiple linear regression. Age, sex, body mass index, alcoholic status and diet did not have any statistically significant effect on daily dose. The observations of genotype frequencies followed Hardy Weinberg equilibrium.

ABCB1 2677G>T is a tri-allelic variant with T and A alleles. In a population, study since a prevalence of A allele was low, its effects were not studied [11]. Patients with GT and TT genotypes required 17.6% higher median maintenance dose per week compared to patients with GG genotype. The increased requirement of warfarin is comparable to other data reporting a 21% increase in dose requirement among T allele patients reflecting the form of a haplotype. The difference between the percentage increase can be due to the involvement of 1236G>T [4].

ABC transporters are present in the biliary canalicular membrane of hepatocytes. Transporters such as MDR1, MDR2, MRP2, BCRP, BSEP, etc. are involved in efflux of drugs and metabolites from liver to bile.

Table 2. Warfarin maintenance dose requirement between genotype groups of *ABCB1 2677G>T*.

Co-dominant model	GG (*n* = 71)	GT (*n* = 91)	TT (*n* = 48)
Dose (mg/day)	5 (3.6–6.4)	6.1 (5–7.5)	5.9 (3.9–7.5)
Dominant model	GG (*n* = 71)	GT + TT (*n* = 139)	
Dose(mg/day)	5 (3.6–6.4)	6.1 (5–7.5)	

N = 210. Values expressed as median (IQR). GG vs. GT p <0.05; GG vs. TTp<0.05; GT vs. TT p>0.05. GG vs. (GT + TT); p <0.05.

Studies done by inhibition assays have found that transport of warfarin from liver to bile is mediated by P-glycoprotein and can also be induced by nuclear hormone receptor PXR [12]. The exact mechanism of how the T allele increases the dose requirement of warfarin is unknown. Possibly, the homozygous T allele may cause an increased P-glycoprotein (P-gp) expression in canalicular membrane compared to the wild-type G allele. This could lead to greater efflux of the drug from hepatocytes and so increase the dose required to achieve the therapeutic INR. ABCB 1 transporter is also expressed in the intestine, where it expels multiple drugs including warfarin. Sakaeda et al. found that there is increased expression of P-gp in individuals with 3435TT genotypes compared to GG and GT. They also suggest that this variant may cause a conformational change in the efflux pump altering its substrate specificity [13]. P-gp may be involved in the transport of Vitamin K and other lipid molecules [14]. Therefore, alteration in the transport of vitamin K which can alter the action of warfarin can also play a mechanistic role in this phenomenon.

A previous study in Caucasian population found that those with variant genotypes (GT and TT) attained a stable maintenance dose of acenocoumarol (4-nitrowarfarin) in a shorter time interval compared to patients with wild-type genotype [14]. In Bulgarian patients, ABCB1 2677GG/3435CC haplotype was associated with low dose requirement of acenocoumarol and 2677TT/3435TT and/or 2677GT/3435TT haplotypes were associated with a higher dose [12]. In a Swedish study, with seven ABCB1 halotypes named A to G, 50% of

patients with G haplotype that included variant genotype (GT and TT) required higher mean dose of more than 0.46 mg/kg per day. In haplotype A which included variant genotypes (GT and TT), 31% of the patients required the same higher dose. In haplotype F, 40% of the patients require higher dose of 0.46 mg/kg per day [1]. The actual effect of these genotypes can only be assessed based on haplotype analysis including other relevant SNPs like exon 1 (−12T>C), exon 2 (−1G>A), exon 11 (1199G>A), exon 12 (1236C>T), exon 26 (3396C4T) and exon 26 (3435C>T). Currently, there are different algorithms available for predicting the accurate starting dose for a patient requiring warfarin and also dose change in current drug users [15]. Inclusion of this genotype into the algorithm may further improve the accuracy of dose prediction. An algorithm developed for a Brazilian population included the genotype of ABCB1 and CYP4F2 and there was increase in algorithm's coefficient of determination (R^2) by 2.6% which explained about 3.6% of warfarin dose variability [13].

We accept certain limitations: we did not consider the effects of other relevant genes (e.g. CYP2C9 and VKORC1 and their polymorphisms). Indeed, the overall effect found from the study can be a net effect of other genes not assessed in this study. Increasing the dose without following any algorithms can result in adverse outcomes. In certain genotypes it is not the dose, but the time to reach target is prolonged as in CYP2C9 *1/*3, *2/*2, *2/*3 and *3/*3. This highlights the necessity and explains the rationale behind developing and validating algorithms with clinical and genetic parameters especially for drugs such as warfarin. Future studies are needed to evaluate the combined role of established genetic markers for warfarin such as CYP2C9 and VKORC1, and also the role of newer genetic polymorphisms including that of ABCB1 2677G>T polymorphism on various parameters related to warfarin such as dose requirement, time to achieve target INR and association with warfarin resistance. We also acknowledge potential effects of drugs (Table 1).

This work represents an advance in biomedical science because it links ABCB1 2677G>T genetic polymorphism and the maintenance dose of warfarin for patients who have achieved a stable INR. Incorporation of this polymorphism to algorithms for dose prediction of warfarin may improve the accuracy of dose prediction.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The study was funded by the intramural grant from Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry.

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