Interethnic variability of CYP4F2 (V433M) in admixed population of Roma and Hungarians

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Abstract

Aims: Pharmacogenetic based dosing recommendations are provided in FDA-approved warfarin label for Caucasians. Evidence of notable difference in dosing algorithms of under-represented populations forced us to explore the genetic variability of *CYP4F2* gene in Roma and Hungarian populations.

Patients, methods: 484 Roma, 493 Hungarian untreated subjects were genotyped for the *CYP4F2**3 (rs2108622) variant by PCR-RFLP assay.

Results, Discussion: We firstly report, that frequencies of the *CYP4F2* rs2108622 GG, GA, AA genotypes and A allele in the Roma population were 46.5 %, 42.6%, 10.9% and 32.2%; in Hungarians 50.1%, 42.2%, 7.7% and 22.8%, respectively. Bearing of two minor alleles of *CYP4F2* missense variant (AA genotype) modestly explains inter-ethnic differences of studied populations ($p < 0.08$). *CYP4F2**3 (V433M) risk allele frequency of Roma (0.32) was in higher range, and of Hungarians (0.23) in lower range, as compared with other world populations.

Conclusions: Roma have an elevated chance for higher mean warfarin dose, besides a decreased risk of major bleeding events in long-term warfarin use.

Keywords: *CYP4F2**3 (rs2108622, V433M), population pharmacogenetics, Roma, Hungarian

Introduction

Inter-ethnic variation of *CYP2C9* and *VKORC1* pharmacogenes, the major genetic determinants of drug response variability in anticoagulant treatment (Cavallari, 2012; Verhoef *et al.*, 2014), have been described already in Roma and Hungarian populations (Sipeky, Csongei, *et al.*, 2009; Sipeky, Lakner, *et al.*, 2009). *Cytochrome P450 4F2 (CYP4F2)*, as additional candidate gene, supposed to have influence on anticoagulant therapy predominantly in minor ethnic populations (Danese *et al.*, 2012; Daly, 2013).

CYP4F2 by metabolizing vitamin K limits its availability and counteracts the effects of *VKORC*. *CYP4F2* rs2108622 (c. G1297A, p. V433M, *CYP4F2**3 haplotype) minor allele results in higher mean warfarin dose (Caldwell *et al.*, 2008; Takeuchi *et al.*, 2009; Cen *et al.*, 2010). Studies also confirmed, that *CYP4F2* V433M variant explains significant amount of inter-individual warfarin dose variance depending on the ethnic background: 1.5% in Swedes (Takeuchi *et al.*, 2009), 4% in Han Chinese (Cen *et al.*, 2010), 7% in Italians (Borgiani *et al.*, 2009) and 2% in North Americans (Caldwell *et al.*, 2008). These results could not be revealed in ethnically more diverse, admixed populations like the Brazilians (Perini *et al.*, 2010), African-Americans (Cavallari *et al.*, 2010), Egyptians (Shahin *et al.*, 2011), where the rs2108622 minor allele frequency appears considerably lower, combined with its small effect size.

The minor allele at rs2108622 is also associated with increased blood pressure (Ward *et al.*, 2014), and in contrast, with decreased vitamin E metabolism (Major *et al.*, 2011). Recent studies report association with ischemic stroke (IS) in the Han Chinese (Yan *et al.*, 2015), and 40% lower risk of bleeding events in a mainly white Caucasian cohort (Roth *et al.*, 2014).

Importance of *CYP4F2* gene in pharmacogenetics is underlined by the clinical annotation for *CYP4F2* rs2108622 testing. According to the warfarin dosing guideline of Clinical Pharmacogenetics Implementation Consortium (CPIC) the *CYP4F2* gene is marked as gene of potential importance, and has an 1B clinical annotation level of evidence, which means that variant-drug association exist, however it must be replicated in more under-represented cohorts (Johnson *et al.*, 2011). Genotype information of *CYP4F2**3 functional SNP, in addition to a number of other variables like the ethnicity, is already incorporated in warfarin dosing algorithm (<http://www.warfarindosing.org/Source/Home.aspx>).

In spite of these facts, *CYP4F2**3 allele and genotype distribution among racial multi-ethnic populations have not been systematically established. Heterogeneous origin, genetic composition of the colourful Roma (Gypsy) and unique Hungarian populations serve as a

background for pharmacogenetic studies (Moorjani *et al.*, 2013; Lazaridis *et al.*, 2014). In order to implement translational personalized medicine in under-represented minority groups, we investigated the allele and genotype frequencies of *CYP4F2**3 allele in randomly selected Roma and Hungarian individuals.

Materials and methods

Study population

The DNA samples were from the central Biobank of the University of Pecs that is part of the National Biobank Network of Hungary (www.biobank.hu), as well as the pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) (<http://www.bbmri.eu/bbmri/>). Governance principles and maintenance management of the Biobank had been approved by the Hungarian National Research Ethics Committee. During the collection and analysis of DNA samples and processing of the accompanying clinical and personal data the guidelines and regulations of the Helsinki Declaration in 1975. and the currently operative National regulations were followed. DNA of total of 484 randomly selected healthy Roma samples (230 males and 254 females, mean age 51 ± 20 years, range: 18-91 years) and 493 randomly selected healthy Hungarian (278 males and 215 females, mean age 37 ± 13 years, range: 18-64 years) were used in the study.

Molecular methods

Genomic DNA was isolated from peripheral leukocytes using routine salting out method, and was stored with patient's informed consent. The *CYP4F2**3 (rs2108622, c.G1297A, V433M, exon 2) polymorphisms of the *CYP4F2* (MIM*604426, Chr 19) gene has been analysed. For primer design the sequences deposited into the GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) were used.

Genotyping was carried out using polymerase chain reaction (PCR) followed by restriction endonuclease digestion (RFLP). For detection of the c. G1297A polymorphism the following primers were used: 5'- ATCAACCCGTTCCACCT -3' and 5'- ACATTGTGCTCCCAGACG-3'. PCR amplification was carried out in a final volume of 50 μ l on an MJ Research PTC 200 thermal cycler. PCR conditions were as follows: pre-denaturation for 2 min at 96°C, followed by 35 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec by primer specific temperature (52°C), primer extension for 30 sec at 72°C, final extension at 72°C for 5 min.

Ten µl PCR product of the c. G1297A was digested by PvuII restriction enzyme. The digested PCR product was separated by electrophoresis using a 3% agarose gel stained with ethidium-bromide and visualized by an UV transilluminator. In the samples with c.1297 GG genotype the PvuII cleaves the 492-bp long PCR product in 176-bp and 316-bp fragments. While in AA homozygotes the recognition site of the enzyme was missing, resulting in no digestion. However, all the homozygote variant samples from the PCR-RFLP assay were direct sequenced with the same primers utilizing an ABI PRISM 3100 AVANT genetic analyser.

Statistical analysis of data

Statistical significance ($p < 0.05$) was assessed by χ^2 test to compare the differences between studied groups. Statistical analyses were performed applying Excel for Windows and SPSS 11.5 package for Windows (SPSS Inc., Chicago, IL).

Results and Discussion

This is the first study on functional *CYP4F2* (V433M) missense variant in the world-dispersed Roma population, compared to Hungarians. The allele and genotype frequencies of the *CYP4F2**3 (c. G1297A) functional polymorphism in two examined populations compared to data available from other world populations are shown in Table 1. Allele and genotype frequencies were in Hardy-Weinberg equilibrium both in Roma and Hungarian subjects.

The frequencies of the *CYP4F2* rs2108622 GG, GA, AA genotypes and A variant allele in the Roma population were 46.5 %, 42.6%, 10.9% and 32.2%, and in Hungarians 50.1%, 42.2%, 7.7% and 22.8%, respectively. The comparisons of frequencies between Roma and Hungarians revealed marginal ethnic difference reflected by AA genotype carriers ($p < 0.08$).

Considerable result of our study, that more than half of the Roma population minority has a variant A allele (GA+AA, 53.5%), and the Hungarians have also high carrier allele frequency (GA+AA, 49.9%), predicting higher daily mean warfarin dose requirement compared to ancestral G allele carriers.

CYP4F2 rs2108622 genotype and allele frequencies are highly dependent from the demographic past of the population; *CYP4F2**3 prevalence reported from ethnic populations is summarized in Table 1. Comparing the interethnic differences of rs2108622 polymorphisms can be concluded, that the lowest minor allele frequency (8%) is found in

African-Americans, the highest in Indians (44%) from Singapore (Singh *et al.*, 2011). The rs2108622 SNP is relatively common in Egyptians (42%) (Shahin *et al.*, 2011) and in Spanish white people (38%) (Pérez-Andreu *et al.*, 2009). In contrast, the studied polymorphism has relatively low frequencies in Malays (14%) (Singh *et al.*, 2011) and in Black Brazilians (15%) (Perini *et al.*, 2010). Noteworthy, Indians have 2-3 times higher A allele frequency compared to other orientals, like Chinese and Malays, from the same geographic area of Singapore (Singh *et al.*, 2011). The minor allele frequency of *CYP4F2* polymorphism found in Roma population (32.2%) was in upper range of observed Caucasian populations (23.3-38.0%), and was much lower than in Indians from Singapore (44%) (see Table 1.). The prevalence of *CYP4F2**3 variant allele in Hungarian population (22.8%) is the lowest among Caucasians.

Conclusions

Functional conclusions from the study are the followings. The risk allele frequency of Roma (0.32) was in higher range, and of Hungarians (0.23) in lower range, as compared with other world populations. In addition, carrier allele frequencies of Roma (53.5%) also suggest the need for higher daily mean warfarin dose requirement compared to ancestral G allele carriers, which proposed to be incorporated in further dosing guidelines. From the pharmacogenetic point of view, Roma from Hungary may have elevated risk for under-anticoagulation, in contrast decreased risk of major bleeding events in long-term warfarin use. However, they do not seem to raise marked public health concerns comparatively to Hungarians.

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Table 1. Distribution of genotype and allele frequency of *CYP4F2**3 (V433M) functional variant in Roma and Hungarian samples compared to other population data

Population	n	<i>CYP4F2</i> *3 (rs2108622) (%)					Reference
		GG	GA	AA	GA+AA	A allele	
Roma	484	46.5	42.6	10.9	53.5	32.2	Current study
Hungarian	493	50.1	42.2	7.70	49.9	22.8	Current study
Slovak	112	47.0	49.0	4.00	53.0	29.0	(Krajčiová <i>et al.</i> , 2014)
Norwegian	212	58.0	34.9	7.10	42.0	24.5	(Kringen <i>et al.</i> , 2011)
Finnish	3325	63.8	31.0	5.20	36.2	20.7	www-sisuproject .fi
1000 Genome_EUR	503	51.9	38.2	9.90	48.1	29.0	www.1000genomes.org
Caucasian	300	42.7	46.3	11.0	57.3	34.2	(Scott <i>et al.</i> , 2010)
Caucasian	141	46.0	42.0	12.0	54.0	32.0	(Borgiani <i>et al.</i> , 2009)
French	283	48.8	43.6	7.60	51.2	29.4	(Pautas <i>et al.</i> , 2010)
Hispanic	300	58.7	36.0	5.30	41.3	23.3	(Scott <i>et al.</i> , 2010)
Spanish (White)	100	41.0	42.0	17.0	59.0	38.0	(Pérez-Andreu <i>et al.</i> , 2009)
Asian	300	48.0	43.0	9.00	52.0	30.5	(Scott <i>et al.</i> , 2010)
Asian	124	66.9	31.5	1.60	33.1	17.0	(Singh <i>et al.</i> , 2011)
Japanese	440	53.0	39.8	7.20	47.0	27.2	(Cha <i>et al.</i> , 2010)
Han-Chinese	115	41.7	47.8	10.4	58.2	34.4	(Liang <i>et al.</i> , 2012)
Han-Chinese	222	52.0	41.0	7.00	48.0	27.0	(Cen <i>et al.</i> , 2010)
Chinese	88	67.0	28.4	4.50	32.9	19.0	(Singh <i>et al.</i> , 2011)
Malay	88	73.9	23.8	2.20	26.0	14.0	(Singh <i>et al.</i> , 2011)
Indians	88	32.9	45.4	21.5	66.9	44.0	(Singh <i>et al.</i> , 2011)
Egyptian	195	35.4	45.3	19.3	64.6	42.0	(Shahin <i>et al.</i> , 2011)
Ashkenazi Jewish	500	43.4	47.6	9.00	56.6	32.8	(Scott <i>et al.</i> , 2010)
African-American	50	84.0	16.0	0.00	16.0	8.00	(Singh <i>et al.</i> , 2011)
African-American	300	78.0	20.7	1.30	22.0	11.7	(Scott <i>et al.</i> , 2010)
Brazilian (White)	184	50.0	39.0	11.0	50.0	30.0	(Perini <i>et al.</i> , 2010)
Brazilian (Mixed)	112	64.0	26.0	10.0	36.0	23.0	(Perini <i>et al.</i> , 2010)
Brazilian (Black)	74	72.0	27.0	1.00	28.00	15.0	(Perini <i>et al.</i> , 2010)

n = number of subjects

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