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Interethnic differences of CYP2C9 alleles in healthy Hungarian and Roma population samples: Relationship to worldwide allelic frequencies

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ABSTRACT

CYP2C9 gene polymorphisms are widely studied in several ethnic groups, however they are less known in the Roma population. The aim of this work was to study the ethnic differences of the CYP2C9 allele distribution in a healthy Roma population in order to compare them with a healthy Hungarian population. A total of 535 Hungarian and 465 Roma volunteers were genotyped for the CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) allelic variants by PCR-RFLP assay. The frequencies of the CYP2C9*1, *2 and *3 alleles in the Hungarian population were 0.787, 0.125, and 0.088 and in Roma 0.727, 0.118, and 0.155, respectively. We found a significant difference in CYP2C9*3 prevalence between the Hungarian and Roma populations, which have therapeutic consequences ($p<0.005$). The distribution of *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 genotypes in Hungarians were 0.620, 0.195, 0.139, 0.021, 0.015, and 0.011, while in Roma were 0.533, 0.168, 0.219, 0.011, 0.047, and 0.022, respectively. A significant difference was found between the Hungarian and Roma populations regarding the *1/*1, *1/*3 and the *2/*3 ($p<0.005$) genotypes. This is the first study to investigate the polymorphisms of CYP2C9 gene in the two largest populations in Hungary, healthy Hungarians and Roma. The prevalence of variant CYP2C9 alleles in the Hungarian population is similar to that observed in other European populations. In contrast, the Roma population differs from Hungarians, from most of other Caucasian groups, and from Indians in the incidence of CYP2C9 common variants. The difference in allele distribution patterns between the two populations studied has therapeutic implications as it influences the optimization of therapies.

Introduction

Cytochrome P450 (CYP) 2C9 is one of the most important enzymes in human drug metabolism and its genetic polymorphisms are known to contribute to interindividual and interethnic variations in the metabolism of several drugs in humans [1–3]. CYP2C9 is involved in the metabolism of 10–20% of all drugs, including many clinically important pharmaceuticals such as coumarin anticoagulants, losartan, tolbutamide, sulfonylurea drugs, angiotensin II blockers, nonsteroidal anti-inflammatory drugs (NSAIDS) and phenytoin, some with narrow therapeutic index [3–10]. In addition to the wild-type allele (CYP2C9*1), a wide number of genetic variants have been described including the two variants most common in Caucasians, the CYP2C9*2 (C430T, Arg144Cys in exon 3) and the CYP2C9*3 (A1075C, Ile359Leu in exon 7) variants, encoding enzymes with reduced enzymatic activity [7,11,12]. Several studies show that *3 is associated with a lower intrinsic clearance of substrate drugs than *2; and CYP2C9*3 has less than 5% of the activity of the wild-type enzyme, whereas CYP2C9*2 has about 12% of that activity [12–14]. However, the extent of reduction in catalytic activity caused by each variant is substrate specific [15].

Currently, the total Roma population size is estimated to be about 12 million in the world, and 8–10 million Gypsies live in Europe today [16,17]. Hungary has the fourth largest Roma population in Europe, considering the size of the Roma population of about 700,000 people [17]. The population of Hungary is comprised largely of Hungarians, however, many ethnic minorities also reside here, with the Roma forming the largest group [18]. Besides the considerable size of the Roma population in Hungary, the different origin of Roma and Hungarians from neighboring populations in Europe is also important. Evidence has been presented that Roma people are of Indian origin [16,17,19–23]. The Hungarians are also unique among the other European populations because of the ancestry of the ancient Magyars, who had come from the eastern side of the Ural Mountains [24,25].

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Thus the Roma and Hungarians are both different from other European Caucasian populations in their origin [16,17,19–22,24,25].

As the allelic variants of human cytochrome P450 CYP2C9 gene vary in frequency among different ethnic groups [7,26], we determined the major allelic variants of CYP2C9 gene in randomly selected healthy Hungarian and Roma population subjects, originated from Hungary, and to compare them with results obtained in other ethnic populations.

Materials

Study population

The study was done using DNA from healthy Roma and healthy Hungarian subjects. The Roma samples used are from a relatively homogenous subpopulation in North-East Hungary. Hungarian Caucasian samples are from different regions of Hungary. During personal interviews, the Hungarians did not assign themselves to any minor ethnic groups living in Hungary, while the Roma people declared their Roma origin.

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/). The 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 535 healthy Hungarian (251 males and 284 females, mean age 49 ± 16 years, range: 20–92 years) and 465 healthy Roma samples (300 males and 165 females, mean age 40 ± 16 years, range: 18–90 years) were used in the study.

Molecular methods

In order to genotype the samples we applied PCR/RFLP assays to characterize the two allele-tagging SNPs. For primer design of CYP2C9*2 (rs1799853) variant the sequence deposited into the GenBank was used. The CYP2C9*2 (Arg144Cys) mutation was detected using the following forward and reverse primers 5′-GGGAGGATGGAAAACAGAGACTT-3′ and 5′-GGTCAGTGATATGGAGTAG GGTCA-3′, respectively. PCR amplification was carried out in a final volume of 50 μl on an MJ Research PTC 200 thermal cycler. PCR conditions were as follows: predenaturation for 2 min at 95 °C, followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, primer extension for 30 s at 72 °C, and the final extension at 72 °C for 5 min. 10 μl PCR product was digested by 1 U Cfr13I (AsuI) restriction enzyme. The digested PCR products were separated by electrophoresis using a 3% agarose gel stained with ethidium bromide and were visualized by UV illumination. In the amplicons there was an obligatory cleavage site to enable us to monitor the efficacy of the digestion. In samples with CYP2C9*2 CC genotype the Cfr13I cleaves the 309 bp long PCR product into 48 bp, 84 bp and 177 bp fragments. If the CYP2C9*2 430T allele was present in homozygous form 48 bp and 261 bp fragments could be detected. Genotyping of CYP2C9*3 (Ile359Leu) polymorphism was performed as previously described by Sullivan-Klose et al., as this method gave highly specific amplification of the CYP2C9 gene confirmed by DNA sequencing [13,27,28]. For random control of both the assays we used direct sequencing with the same primers utilizing an ABI PRISM 3100 AVANT genetic analyser.

Statistical analysis of data

We applied Chi-square test (nonparametric test for discrete variables) to compare the differences between the two groups studied. The value of $p<0.05$ was considered as statistically significant. Statistical analyses were performed applying Excel for Windows and SPSS 11.5 package for Windows (SPSS Inc., Chicago, IL).

Table 1
Allele and genotype frequencies and the predicted phenotype of CYP2C9 in the healthy Hungarian and Roma population samples; data are compared with those reported for Indian and Caucasian populations.

CYP2C9	Current study		Reported data	
	Hungarian $n=535$	Roma $n=465$	Indian [38] $n=135$	Italian Caucasian [26] $n=360$
Allele frequency				
*1	0.787	0.727[a,b]	0.907	0.778
*2	0.125	0.118[b]	0.026	0.125
*3	0.088	0.155[a,b,c]	0.067	0.097
Genotype frequency				
*1/*1	0.620	0.533[a,b,c]	0.823	0.619
*1/*2	0.195	0.168[b]	0.044	0.172
*1/*3	0.139	0.219[a,b,c]	0.127	0.145
*2/*2	0.021	0.011	ND	0.028
*2/*3	0.015	0.047[a,b]	0.007	0.022
*3/*3	0.011	0.022	ND	0.014
wt/wt (EM)	0.620	0.533[a,b,c]	0.823	0.619
wt/mut (IM)	0.334	0.387[b,c]	0.171	0.317
mut/mut (PM)	0.047	0.080[a,b]	0.007	0.064

No significant difference was observed between Hungarian and Caucasian populations considering the CYP2C9 gene.
n, number of subjects;
wt/wt, homozygous wild-type;
wt/mut, heterozygous mutant;
mut/mut, homozygous mutant;
EM, extensive metabolizer;
IM, intermediate metabolizer;
PM, poor metabolizer.
[a] $p<0.03$, when Roma are compared with Hungarian population.
[b] $p<0.04$, when Roma are compared with Indian population.
[c] $p<0.04$, when Roma are compared with Caucasian population.

Results

The distribution of CYP2C9*1, *2, and *3 alleles as well the *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 genotypes in Hungarian and Roma populations is presented in Table 1. All CYP2C9 allele and genotype frequencies were in Hardy–Weinberg equilibrium both in Roma and in Hungarian subjects. Besides the wild-type allele, the CYP2C9*2 was the most common allele identified in Hungarians, whereas in the Roma population the CYP2C9*3 was most frequent. In addition, we found a significant (1.8-fold) increase in CYP2C9*3 prevalence in Roma population compared to Hungarian samples ($p<0.001$). Furthermore, the frequency of *1/*3 genotype observed was considerably higher in the Roma group than in Hungarians (0.219 versus 0.139, $p<0.001$). The *1/*1 genotype in the Hungarian population was more common than in Roma subjects ($p<0.005$). Based on the distribution of CYP2C9 gene variants, the proportion of subjects homozygous for the wild-type allele (genotypically identified as extensive metabolizer, EM) was higher in Hungarians ($p<0.005$), while subjects carrying two detrimental alleles (with impaired enzyme activity, poor metabolizer, PM) are more frequent in Roma population ($p<0.03$).

Discussion

Much of the interindividual and interethnic differences in the effects of drugs are attributed to genetic differences in their metabolism and utilization [29]. A number of drugs are metabolized by the human cytochrome P450 system [7]. CYP2C9 is the most abundant of the CYP2C enzymes [30]. It is involved in the metabolism of more than 100 drugs [8,10]. The metabolism of S-enantiomers of coumarins, warfarin, acenocoumarol and phenprocoumon is significantly decreased by the presence of both the CYP2C9*2 and CYP2C9*3