GENETIC VARIABILITY AND INTERETHNIC DIFFERENCES OF SELECTED PHARMACOGENETICALLY RELEVANT GENES IN AVERAGE HUNGARIAN AND ROMA POPULATION SAMPLES

Doctoral (Ph.D.) thesis

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1. **INTRODUCTION**

Variation of genetic variability at the pharmacogenetically relevant genes is the most important cause of variable drug response. Ethnic differences in drug response are well known, thus the optimal drug dose vary between populations of different origin. The population of Hungary is comprised largely of Hungarians, however, many ethnic minorities also reside here, with the Roma forming the largest group. It is well known, Roma minorities live all around the world, but the genetic profile of this minority is less studied. The Roma differ from all the populations of countries they live because of their origin. Evidences have been presented that Roma people are of Indian origin, and thus have different genetic structure than people of Caucasian origin. On the historical basis Hungarians are from the eastern side of the Ural Mountains, thus the ancestry of the ancient Magyars is also differs from the Europeans. Thus the different origin of Roma and Hungarians from neighbouring populations in Europe is important in the clinical therapy they receive.

Cytochrome P450 system plays a key role in the drug metabolism. Cytochrome P450 (CYP) 2C9 [MIM 601130] 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. CYP2C9*1 is the wild-type allele, and besides there are two important single nucleotide polymorphisms, the CYP2C9*2 (C430T, exon 3) associated with a functionally important Arg144Cys substitution and the CYP2C9*3 (A1075C, exon 7) associated with another important Ile359Leu substitution. Both variants are encoding enzymes with reduced enzymatic activity. The CYP2C9 polymorphisms are important determinants of the warfarin dose. The allelic variants of human cytochrome P450 CYP2C9 gene vary in frequency among different ethnic groups.

VKORC1 [MIM 608547] is the key enzyme of the vitamin K cycle and the molecular target of coumarins. Genetic variations of VKORC1 gene can greatly affect the individual response to coumarins. The major natural VKORC1 haplotypes are determined by the combinations of the G-1639A, G9041A, C6009T haplotype tagging SNP-s of VKORC1 gene. With this approach the ancestral VKORC1*1, and three further major haplotypes, the VKORC1*2, *3, and *4 can be differentiated. Rieder et al. identified a low-dose haplotype group (A) and a high-dose haplotype group (B). The mean maintenance dose of warfarin differed significantly among the haplotype group combinations. VKORC1 haplotype groups A and B explained approximately 25% of the variance in dose. In conclusion, VKORC1 genotype was found to determine 25-40% of individual coumarin dose requirement.

P-glycoprotein (P-gp), functions as an energy-dependent drug-transport pump and it is responsible for the multidrug resistance in cancer cells. P-gp plays an important role in the bioavailability of a wide variety of drugs, including warfarin. P-gp is the product of the human multidrug resistance 1 (MDR1/ABCB1) gene. MDR1 [MIM 171050] gene is highly polymorphic. Among the MDR1 SNPs many researchers focused on C3435T variant. Further studies revealed that C3435T SNP was closely linked to other common polymorphisms, such as C1236T and G2677A/T. It has been reported, that the distribution of the C3435T, C1236T, 2677A/T polymorphisms is significantly influenced by ethnicity. Using the three common exonic polymorphisms enabled us to establish the haplotype profile of the Roma and Hungarian populations. Some studies revealed that MDR1 haplotypes differed greatly between ethnic groups. In summary, knowledge of genetic variability of ABC transporters between different ethnic groups is relevant pharmacological factor that can be used to understand variability in drug response.

2. OBJECTIVES OF THE WORK

Although the polymorphisms of CYP2C9, VKORC1 and MDR1 genes are well documented in several populations, there is no report considering the Roma and Hungarian populations. In this way the main purposes of the work are:

- To determine the allele frequencies of functionally significant polymorphisms of clinically important DME, the CYP2C9, in healthy Hungarian and Roma populations.

- To characterize the allele frequencies of the drug target, VKORC1, in healthy Hungarian and Roma population subjects.

- To describe the allele frequencies of most relevant SNP-s of the MDR1 drug transporter in healthy Hungarian and Roma population subjects.

- To establish the haplotype profiles of both the VKORC1 and MDR1 genes in healthy Hungarian and Roma population samples.

- To compare the allele and haplotype frequencies of CYP2C9, VKORC1 and MDR1 genes of the Hungarian and Roma populations with results available for other ethnic populations in literature, mainly with Caucasian and Indian populations.

- To give useful informations in connection with the origin of the Hungarian and Roma populations.

3. MATERIALS AND METHODS

The study was done using DNA from healthy Roma and healthy Hungarian subjects. During personal interviews, the Hungarians did not enroll themselves to any minor ethnic groups living in Hungary. In CYP2C9 study DNA of total of 535 healthy and 465 healthy Roma samples were used. A total of 510 healthy Hungarian DNA samples and 451 samples collected from Roma people were used for the VKORC1 study. In MDR1 study DNA of total of 503 healthy Hungarian and 465 healthy Roma samples were used.

Genomic DNA was isolated from peripherial leukocytes using routine salting out method. In order to genotype the samples we applied PCR/RFLP assays to characterize the polymorphisms of studied genes.

The CYP2C9*2 (Arg144Cys) mutation was detected using the following forward and reverse primers 5'-GGGAGGATGGAAAACAGAGACTT-3' and 5'-GGTCAGTGATATGGAGTAG GGTCA-3', respectively. PCR product was digested by 1U Cfr13I (AsuI) restriction enzyme. Genotyping of CYP2C9*3 (Ile359Leu) polymorphism was performed as previously described by Sullivan-Klose et al..

For determination of the c.-1639 G/A polymorphism (rs9923231) the following primers were used: 5'-ATCCCTCTGGGAAGTCAAGC-3', and 5'-CACCTTCAACCTCTCCATCC-3'. To test the 9041G/A (rs7294) SNP the 5'-TTTAGAGACCCTTCCCAGCA-3' and 5'-AGCTCCAGAGAAGGCAACAC -3' oligonucleotides were used. For the amplification of the target sequence of C6009T (rs17708472) the 5'-AGGCGTTAGCATAATGACGG -3' and 5'-GGGTGGAACCAGGTTAGGAC-3' primers were utilized. The amplicon of VKORC1 3673 G/A SNP was digested with BcnI endonuclease. The SsiI was used to cleave the PCR product of VKORC1 G9041A variant, and the FspBI the PCR product of C6009T polymorphism.

For detection of the C1236T (rs1128503) polymorphism the following primers were used: 5'-AGCTATTCGAAGAGTGGGCA-3', and 5'-GTCTAGCTCGCATGGGTCAT-3'. The G2677T/A (Ala893Thr/Ser) (rs2032582) SNP was detected using two set of primers: 5'-GGTTCCAGGCTTGCTGT AAT-3' (1) forward, 5'- TTTAGTTTGACTCACCTTCCCTG-3' (1) reverse, and 5'-CAGCATTCTGAAGTCATGGAA-3' (2) forward, 5'-GTCCAAGAACTGGCTTT GCT-3' (2) reverse. For the amplification of the target sequence of C3435T (rs1045642) the 5'-GATGTCTTGTGGGAGAGGGA-3' and 5'-GCATGTATGTTGGCCTCCTT-3' primers were utilized. 10 µl PCR product of the C1236T, G2677T/A (1), G2677T/A (2) and C3435T primers was digested by BsuRI, HpyCH4V, RsaI and MboI restriction enzymes, respectively. Approximately 10% of the total PCR products were selected randomly for direct sequencing to confirm the results obtained by PCR-PFLP procedure by an ABI PRISM 3100 AVANT Genetic Analyser.

We used the 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).

4. RESULTS

4.1. CYP2C9 gene

The distribution of CYP2C9*1, *2, *3 alleles as well the *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, *3/*3 genotypes in Hungarian and Roma populations are presented in Table 1. All CYP2C9 allele and genotype frequencies were in Hardy-Weinberg equilibrium both in Roma and in Hungarian subjects. Beside the wild-type allele, the CYP2C9*2 was the most common allele identified in Hungarians, while in the Roma population the CYP2C9*3 was the most frequent. In addition, we found a significant (1.8-fold) increase in CYP2C9*3 prevalence in Roma population compared to Hungarian samples, which has therapeutic consequences ($p<0.001$). Furthermore, the frequency of *1/*3 genotype observed here was considerably higher in the Roma group than in Hungarians (0.219 vs. 0.139, $p<0.001$). Interestingly, 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 ($p<0.03$).

4.2. VKORC1 gene

All VKORC1 allele frequencies were in Hardy-Weinberg equilibrium both in Hungarian, and in Roma subjects. The frequency of allelic variants and genotypes of VKORC1 tagging polymorphisms in the Roma group and Hungarians are summarized in Table 2.

For the G-1639A polymorphism significant differences were observed in the prevalence of homozygous GG and AA genotypes, GA+AA carriers, and in minor allele frequency between the Roma and Hungarian samples ($p<0.001$); for the G9041A SNP exactly the same distribution patterns could be detected. By contrast, the genotype and allele distributions for the C6009T SNP did not differ between Roma and Hungarians (Table 2.).

In the Hungarian population sample (Table 3.) the haplotypes in decreasing order of their frequencies were the *2 (39%), *3 (37%), *4 (21%) and *1 (3%), while in the Roma population samples *3 (46%), *2 (30%), *4 (19%), and *1 (5%). The statistical analysis revealed significant difference in the prevalence rate of VKORC1*2 and VKORC1*3 haplotypes between the Roma and average Hungarian population ($p<0.005$).

By using the above VKORC1 haplotypes we could determine the VKORC1 genotypes of each subject in the studied populations (Table 4.).

The ancestral VKORC1 *1/*1 genotype could be found both in Roma and in Hungarians. This ancestral genotype was also detected in combination with other genotypes (*1/*2, *1/*3, *1/*4), but the *1/*4 was not detectable in Hungarian population. Comparing the Roma with the Hungarians significant difference was observed in prevalence of *1/*4, *2/*2,*2/*4 and *3/*3 genotypes ($p<0.04$).

4.3. MDR1 gene

The allele and genotype frequencies of studied MDR1 polymorphisms in the Hungarian group and Roma are shown in Table 5.

The allele and genotype frequencies of studied MDR1 SNPs did not show a significant deviation from Hardy-Weinberg equilibrium neither in Roma nor in Hungarian subjects.

Considering the MDR1 C1236T polymorphism, a significant difference was observed in the presence of CC (20.7 vs. 33.2%) and TT (30.8 vs. 21.9%) genotypes, the CT+TT (79.4 vs. 66.8%) carriers and the T allele frequency in Roma compared to Hungarians ($p<0.002$), respectively. The 1236C (0.557) was the most common allele identified in Hungarians, while in Roma population the 1236T (0.551) allele was most frequent. By contrast, no significant difference was observed between Roma and Hungarian populations considering the G2677T/A polymorphism. However, subjects carrying two of mutated alleles (TA) are two-times more common in Roma population than in Hungarians (1.3 vs. 0.6%). The frequency of the 2677A allele was almost two-fold higher in Roma than in Hungarian group, however the difference did not reach the statistical significance level (0.020 vs. 0.011, $p=0.078$). In MDR1 exon 26 (C3435T), higher frequency of the T allele was observed in Hungarians compared with Roma (0.527 vs. 0.482, $p<0.05$).

Subsequent analyses of the MDR1 haplotype frequencies, estimated from the genotype data, were compared between the two studied groups (Table 6.).

There were 12 possible MDR1 haplotypes and the frequencies of these were statistically different between the Roma and Hungarian populations. All 12 possible haplotypes were observed in Roma, compared with 11 haplotypes in Hungarians. The 1236T/2677A/3435C haplotype was not detectable in Hungarian population. The two most frequent MDR1 haplotypes both in Roma and Hungarian populations were TTT (36.0 vs. 37.5%) and CGC (35.3 vs. 41.4%). The haplotypes TTT, CGC, TGC, TTC, CGT occured at high frequencies in Roma population (6.02-36.0%), whereas in Hungarians TTT, CGC and CGT were the most common identified haplotypes. The statistical analysis revealed significant difference in the prevalence rate of CGC, TGC, TTC, CGT and CTT haplotypes between the healthy Roma and Hungarian populations ($p<0.009$). The occurrence of 1236T/2677G/3435C haplotype was four-fold higher in Roma than in Hungarians. In addition, the presence of 1236T/2677T/3435C haplotype was three-fold higher in Roma than in Hungarians. Whereas the frequency of 1236C/2677T/3435T haplotype was two-fold higher in Hungarian than in Roma group.

Comparison of haplotype profile of both studied groups and other populations is also provided in Table 6. For the Caucasians two sets of data are listed, as there were considerable differences between the two groups found in the literature. The Roma population showed significant differences in TTT, CGC, TGC, TTC, CGT, TGT, CTT, TAT, CAC haplotypes when compared to Caucasians and to the Czech population ($p<0.05$) (Table 6.). However, Roma were more similar to the Indian population and difference could be observed only in TTT, CGC, TTC and CTT haplotypes ($p<0.02$). The haplotype structure of Hungarian population differed also from the Caucasian and Czech populations. Significant difference was found in TTT, CGC, CGT, TGT, CTT, TAT and CAC haplotypes ($p<0.03$). In a previous study of Hungarian acute lymphoblastic leukaemia patients the dominating haplotypes were TTT and CGC, in accordance with our results.

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 (Adithan 2003) n=135**	**Italian Caucasian (Scordo 2004) 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
Phenotype frequency				
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

[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
No significant difference was observed between Hungarian and Caucasian population considering the CYP2C9 gene.
n, number of subjects

Table 2. VKORC1 haplotype tagging SNPs in Roma and Hungarian populations; data are also compared with data reported from India, or deposited into database for Caucasians.

VKORC1 polymorphism	Genotype	Current study		Data from other studies	
		Roma n=451 (%)	Hungarian n=510 (%)	Indian (Lee 2006) n=43 (%)	Caucasian (NCBI) n=22/n=23/n=21 (%)
G-1639A	GG	214 (47.5)	180 (35.3)[a]	36 (83.8)[b]	7 (31.8)
	GA	206 (45.7)	262 (51.4)	4 (9.50)[b]	11 (50.0)
	AA	31 (6.87)	68 (13.3)[a]	3 (6.80)	4 (18.2)[c]
	GA+AA	237 (52.6)	330 (64.7)[a]	7 (16.3)[b]	15 (68.2)
	A allele frequency	0.297 (29.7)	0.390 (39.0)[a]	0.116 (11.6)[b]	0.432 (43.2)
G9041A	GG	132 (29.3)	206 (40.4)[a]	4 (8.10)[b]	10 (43.5)
	GA	220 (48.8)	233 (45.7)	8 (18.9)[b]	11 (47.8)
	AA	99 (22.0)	71 (13.9)[a]	31 (73.0)[b]	2 (8.7)
	GA+AA	319 (70.8)	304 (59.6)[a]	39 (91.9)[b]	13 (56.5)
	A allele frequency	0.463 (46.3)	0.368 (36.8)[a]	0.814 (81.4)[b]	0.326 (32.6)
C6009T	CC	293 (65.0)	319 (62.5)	38 (87.8)[b]	13 (61.9)
	CT	144 (31.9)	170 (33.3)	5 (12.2)[b]	7 (33.3)
	TT	14 (3.10)	21 (4.12)	0 (0.00)	1 (4.80)
	CT+TT	158 (35.0)	191 (37.4)	5 (12.2)[b]	8 (38.1)
	T allele frequency	0.191 (19.1)	0.208 (20.8)	0.058 (5.8)[b]	0.214 (21.4)

www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=9923231
www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=7294
www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=17708472
[a]Hungarian population is compared with Roma; $p<0.001$
[b]Indian population is compared with Roma; $p<0.01$
[c]Caucasian population is comapred with Roma; $p<0.05$

Table 3. Ethnic distribution of VKORC1 haplotype frequencies.

Haplotype identification code	Hungarian (n=510)	Roma (n=451)	European (Geisen 2005) GER (n=200)	Italian (Spreafico 2008) (n=220)	Israeli (Loebstein 2007) (n=99)†	Chinese (Perlegen) CHN (n=24)	African (Perlegen) AFR (n=23)	Americans with origin of (Rieder 2005)			
								European (n=186)†	European (n=119)†	Asian (n=120)†	African (n=96)†
VKORC1*1	0.03 (35)	0.05 (44)	<0.001	0.03 (15)	0.01	<0.001	0.31 (14)	<0.001	<0.001	<0.001	<0.001
VKORC1*2	0.39 (400)[a]	0.30 (269)	0.42 (168)	0.43 (189)	0.41	0.95 (46)	0.14 (6)	0.36 (131)	0.38 (89)	0.89 (213)	0.13 (26)
VKORC1*3	0.37 (373)[a]	0.46 (417)	0.38 (152)	0.36 (158)	0.37	0.04 (2)	0.43 (20)	0.43 (160)	0.35 (83)	0.10 (25)	0.43 (82)
VKORC1*4	0.21 (212)	0.19 (172)	0.20 (80)	0.18 (78)	0.18	<0.01	0.12 (6)	0.21 (77)	0.24 (56)	<0.01 (2)	0.06 (11)

Chinese (www.perlegen.com)
African (www.perlegen.com)
[a]Hungarian population is compared with Roma; $p<0.005$
†Other haplotypes were also identified in low percentile rates.
Haplotype <0.001 means that this haplotype was not found, but its existence cannot be excluded (definition is from Geisen 2005)

Table 4. VKORC1 genotype distribution in Roma, Hungarian and Italian Caucasian population, and the predicted warfarin dose.

VKORC1 genotypes	Predicted dose	Genotype frequency (%)		
		Roma n=451 (%) (healthy)	Hungarian n=510 (%) (healthy)	Italian (Spreafico 2008) n=220 (%) (anticoagulated)
***1/*1**	Ancestral†	1 (0.22)	1 (0.20)	0 (0.00)
***1/*2**	Ancestral/Low†	14 (3.10)	18 (3.53)	3 (1.4)
***1/*3**	Ancestral/High†	18 (3.99)	15 (2.94)	8 (3.6)
***1/*4**	Ancestral/High†	10 (2.22)	0 (0.00)[a]	4 (1.8)[c]
***2/*2**	Low	31 (6.87)	69 (13.6)[a]	48 (21.8)[b,c]
***2/*3**	Intermediate	130 (28.8)	146 (28.6)	60 (27.3)
***2/*4**	Intermediate	63 (14.0)	98 (19.2)[a]	30 (13.6)
***3/*3**	High	99 (22.0)	70 (13.8)[a]	29 (13.2)[b]
***3/*4**	High	71 (15.7)	72 (14.1)	32 (14.5)
***4/*4**	High	14 (3.10)	21 (4.12)	6 (2.7)

†Functional significance of these genotypes is still not clear.
[a]Hungarian population is compared with Roma; $p<0.04$
[b]Italian population is compared with Roma; $p<0.01$
[c]Italian population is compared with Hungarian; $p<0.01$

Table 5. Allele and genotype frequencies of MDR1 in the healthy Hungarian and Roma population samples; data are compared with those reported for Indian and Caucasian populations.

MDR1 SNP	Genotype and allele	Current study		Reported data	
		Roma n=465 (%)	**Hungarian n=503 (%)**	**Indian (Lakhan 2009) n=96,n=101,n=97 (%)**	**German Caucasian (Cascorbi 2001) n=461 (%)**
C1236T	CC	96 (20.7)[a,c]	167 (33.2)	15 (15.6)	158 (34.4)
	CT	226 (48.6)	226 (44.9)	45 (46.9)	227 (49.2)
	TT	143 (30.8)[a,c]	110 (21.9)[d]	36 (37.5)	76 (16.4)
	Carrier	369 (79.4)[a,c]	336 (66.8)	81 (84.4)	303 (65.6)
	T allele frequency	0.551[a,c]	0.443	0.609	0.410
G2677T/A	GG	125 (26.9)[b]	154 (30.6)	14 (13.9)	143 (30.9)
	GT	228 (49.0)	235 (46.7)	48 (47.5)	227 (49.2)
	TT	94 (20.2)	103 (20.5)	26 (25.7)	74 (16.1)
	GA	11 (2.37)	8 (1.59)	4 (4.00)	9 (2.00)
	TA	6 (1.30)[b]	3 (0.60)	9 (8.90)	8 (1.80)
	AA	1 (0.22)	0 (0.00)	0 (0.00)	0 (0.00)
	Carrier	340 (73.1)[b]	349 (69.4)	87 (86.1)	318 (69.1)
	T allele frequency	0.454[b]	0.441	0.540	0.416
	A allele frequency	0.020[b]	0.011	0.064	0.019
C3435T	CC	124 (26.7)[c]	112 (22.3)	24 (24.7)	96 (20.8)
	CT	234 (50.3)	252 (50.1)	40 (41.2)	233 (50.5)
	TT	107 (23.0)[b]	139 (27.6)	33 (34.0)	132 (28.6)
	Carrier	341 (73.3)[c]	391 (77.7)	73 (75.2)	365 (79.1)
	T allele frequency	0.482[a,c]	0.527	0.546	0.539

[a]$p<0.04$, when Roma are compared with Hungarian population
[b]$p<0.02$, when Roma are compared with Indian population
[c]$p<0.03$, when Roma are compared with Caucasian population
[d]$p<0.03$, when Hungarians are compared with Caucasian population

Table 6. Haplotype frequencies derived from C1236T, G2677T/A and C3435T polymorphisms of MDR1 gene in healthy Roma and Hungarian populations

Number of haplotypes	Haplotype	Haplotype frequency n (%)				
		Roma n=465	Hungarian n=503	Czech (Bandur 2008) n=533	Caucasian (Kroetz 2003) n=247	Indian (South) (epilepsy) (Vahab 2009) n=129
1	**TTT**	335 (36.0)[b,c,d]	377 (37.5)[f]	419 (39.3)	101 (41.0)	33 (25.2)
2	**CGC**	328 (35.3)[a,c,d]	416 (41.4)[e,f]	398 (37.3)	91 (37.0)	18 (13.6)
3	**TGC**	68 (7.31)[a,b,c]	17 (1.68)	30 (2.80)	3 (1.00)	14 (11.0)
4	**TTC**	62 (6.67)[a,b,c,d]	21 (2.08)	13 (1.20)	6 (2.50)	8 (6.20)
5	**CGT**	56 (6.02)[a,b]	91 (9.04)[f]	103 (9.70)	29 (12.0)	13 (9.90)
6	**TGT**	37 (3.98)[b,c]	27 (2.68)[f]	22 (2.10)	1 (0.50)	9 (7.10)
7	**CTC**	15 (1.61)	17 (1.68)	27 (2.50)	4 (1.50)	5 (4.20)
8	**CTT**	10 (1.08)[a,b,d]	29 (2.88)[f]	32 (3.00)	3 (1.00)	29 (22.8)
9	**TAT**	7 (0.75)[b,c]	4 (0.39)[e]	ND	ND	ND
10	**CAC**	6 (0.65)[b]	4 (0.39)[e]	17 (1.60)	6 (2.50)	ND
11	**CAT**	3 (0.32)	3 (0.29)	5 (0.50)	3 (1.00)	ND
12	**TAC**	3 (0.32)	ND	ND	ND	ND

ND, not detected
[a]$p<0.009$, when Roma are compared with Hungarian population
[b]$p<0.04$, when Roma are compared with Czech population
[c]$p<0.05$, when Roma are compared with Caucasian population
[d]$p<0.02$, when Roma are compared with Indian population
[e]$p<0.03$, when Hungarians are compared with Czech population
[f]$p<0.02$, when Hungarians are compared with Caucasian population

5. SUMMARY OF NEW OBSERVATIONS

1. According to our results there is a significant increase in CYP2C9*3 prevalence in Roma population compared to Hungarian samples. We found homozygous mutants for the *3 polymorphism in Hungarians and in Roma in a higher range considering the published literature. The proportion of extensive metabolizers is higher in Hungarians, while poor metabolizers are more frequent in Roma.

2. We showed significant difference of VKORC1 G-1639A polymorphism and VKORC1*2 and VKORC1*3 haplotypes between the Roma and Hungarian samples. No considerable difference was observed between Hungarian and Caucasian population for the VKORC1 SNPs and distribution of VKORC1*2, *3, *4 haplotypes. Except of the -1639AA variant significant difference was observed for all VKORC1 SNPs between the Roma and Indian populations, and for VKORC1*1 and VKORC1*2 haplotype frequencies between Roma and European population.

3. We presented a significant difference in the MDR1 C1236T polymorphism, while no difference was observed in the G2677T/A SNP between the Roma and Hungarians, and higher frequency of the 3435T allele was observed in Hungarians. We found difference in the prevalence rate of CGC, TGC, TTC, CGT and CTT haplotypes between the healthy Roma and Hungarian populations, and of CGC, TGC, TTC, CGT, TGT, CTT, TAT, CAC haplotypes between the Roma and Caucasians. Difference could be observed only in TTT, CGC, TTC and CTT haplotypes when comparing Roma to Indians. The haplotype structure of Hungarian population differed in TTT, CGC, CGT, TGT, CTT, TAT and CAC haplotypes from Caucasians.

4. We demonstrated that the CYP2C9, VKORC1 and MDR1 genetic profile of average Hungarian population is relatively similar to that observed in Caucasian populations. Contrarily, the Roma population differs from Hungarians, from most of other Caucasians and from Indians in the incidence of selected pharmacogenetically relevant genes.

6. PUBLICATIONS OF THE AUTHOR

The thesis is based on the following publications

Sipeky C, Csongei V, Jaromi L, Safrany E, Maasz A, Takacs I, Beres J, Fodor L, Szabo M, Melegh B. Genetic variability and haplotype profile of MDR1 (ABCB1) gene in Roma and Hungarian population samples with a review of the literature. Drug Metabolism and Pharmacokinetics (2010). Accepted. **IF: 2.544**

***Sipeky C**, Keri Gy, Kiss A, Kopper L, Matolcsy A, Timar J, Molnar MJ, Nagy L, Nemeth Gy, Petak I, Rasko I, Falus A, Melegh B. Population Pharmacogenomics and Personalized Medicine Research in Hungary: Achievements and Lessons Learned. Current Pharmacogenomics & Personalized Medicine 2010 Sep; 8(3):194-201. Expert Review.*

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Summary:

Impact factor of own publications wich have served as a base for the thesis: 9.338
Impact factor of publicated papers: 31.979
Impact factor of citable abstracts: 67.931