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The effect of the CYP 2C19*2 polymorphism on stroke care

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Clopidogrel is an inhibitor of platelet-aggregation used in the prevention of secondary stroke. The molecule is activated by the cytochrome P450 2C19 (CYP2C19) enzyme. The frequent CYP2C19*2 point mutation causes loss of enzyme function, a decreased (heterozygous form) or blocked (homozygous form) formation of the active molecule. Thus, for a patient harboring a mutated allele, clopidogrel does not provide effective protection against stroke. Multiple drugs inhibit the CYP2C19 enzyme and their simultaneous use with clopidogrel is especially hazardous for patients with genetically decreased enzyme activity. Frequency of the CYP2C19*2 is variable in different populations, highest rates were detected in some Asian groups. In our study the CYP2C19 genotype was determined in one Hungarian sample of 354 stroke patients and 221 healthy controls. Frequency of the minor allele was found to be 12.87% (12.85% in stroke patients, 12.89% in healthy controls). The proportion of the homozygous CYP2C19*2 variant causing total loss of gene function was 1.74%, rate of the heterozygous allele causing reduced enzyme activity was 22.26% in the total population. Our results for the allele frequencies of the CYP2C19*2 gene are similar to those found in other Caucasian populations. In conclusion, the homozygous mutation, causing ineffectiveness of clopidogrel is relatively rare. However, the heterozygous form in which interaction of CYP2C19 inhibitors causes further decrease in the genetically impaired enzyme activity is present in every fifth drug-taking patient. Based on our findings, we would like to emphasize that it is important to adjust individually antiplatelet treatment in ischemic stroke patients and to take into consideration genetic factors as well as drugs taken for comorbid conditions.

Keywords: CYP2C19, clopidogrel, drug metabolism, stroke, drug interaction

With the spread of modern molecular genetic methods and their routine use in medical diagnostics it becomes possible to determine the most optimal treatment modality based on the integration of genetic, environmental factors of the patient and disease characteristics. This way we are able to find personalized treatment for our patients with the most effective drug and with the fewest side effects.

The cytochrome P450 (CYP) enzymes play a role in the metabolism of many drugs. Enzymes belonging to the CYP1, CYP2 and CYP3 groups are responsible for the oxidative biotransformation, degradation, detoxification of exogenous organic molecules (xenobiotics), for their excretion (drugs, carcinogenic agent, alcohol) or their bioactivation. Other groups of CYP enzymes play a role in the metabolism of fatty acids, prostaglandins and steroids. Among CYP enzymes located in human hepatocytes, the CYP2C group metabolizes 20

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percent of known drug molecules. The subgroup of human CYP2C enzymes is composed of four members, the CYP2C8, CYP2C9, CYP2C18 and CYP2C19. Among them, CYP2C9 and CYP2C19 account for the metabolism of xenobiotics. Genes coding for the CYP2C9 and CYP2C19 proteins have polymorphic expression. Single nucleotide polymorphisms (SNP), deletions, insertions and base substitutions modify the amino acid sequence of proteins, shifting the reading frame, causing an early stop codon or a splicing defect. Twenty-one allelic variants of the CYP2C19 gene are known and marked with *1, *2, *3, etc. according to their frequency of occurrence. These allelic variants differ in their substrate metabolizing capacity. Enzyme polymorphisms create four different phenotypes; normal, decreased, intermediate and rapid metabolizer.

The modifying effect of polymorphisms on the activity of CYP enzymes is the most important factor of individual differences in the oxidative metabolism of drugs. Knowing the type of the given polymorphism permits to choose the most appropriate drug and to set the exact effective dosage.

Frequency of occurrence of the polymorphic CYP alleles shows a great ethnical variability. Frequencies of CYP2C19*2 and CYP2C19*3 have been assessed worldwide. Allelic polymorphisms of CYP2C19 is low in Caucasians (12.0% for CYP2C19*2, 3.0% for CYP2C19*3), but high in Chinese (30.0% for CYP2C19*2, 5.0% for CYP2C19*3, see Table I).

Table I. The relative frequencies (%) of the CYP 2C19*1 and CYP 2C19*2 alleles in the different nations

Population	n	CYP 2C19*1	CYP 2C19*2	Reference
Turkish	404	87.6	12	3
Russian	290	8.,3	11.4	9
Belgian	121	90.9	9.1	2
Dutch	765	86.5	13.3	22
Croatian	200	85	15.0	5
Italian	360	88.9	11.1	20
Bolivian	778	92.1	7.8	6
Saudi-Arabians	97	85	15	11
Jewish Israeli	140	84	15	21
South Indian	341	64	35	1.15
North Indian	121	70	30	17
African	922	82.3	17.3	24
African American	108	75	25	11
Chinese Dai	193	66.3	30.3	12
Japanese	140	54	35	16
Filipino	52	53	39	11

Alleles of CYP2C19 with normal enzyme activity are *1A, *1B, *1C, inactive variants are *2B, *3A, *3B, *4, *5A, *5B, *6, *7 and *8, whereas the recently discovered *17 allele has increased activity. The CYP2C19*2 variant harbors the 681G>A mutation in exon 5 which causes an aberrant splicing site by shifting the reading frame of mRNA. The truncated protein causes total loss of enzyme function. This allelic variant is responsible for the loss of

enzyme function in 70 percent of Caucasians with poor metabolizer phenotype. The goal of this study was to estimate the ratio of the defective minor allele CYP2C19*2 in the Hungarian population and to estimate its importance in the stroke care.

Patients and Methods

We investigated the biological samples of 575 Caucasian individuals from the NEPSYBANK for our study. Mean age of the participants was 65±17.36 years. Of them, 373 had ischemic stroke syndrome (181 female, mean age 69±12.77 years; 192 male, mean age 72±15 years) and 202 were healthy controls (145 female, mean age 43±13.12 years; 57 male, mean age 66±14 years). The healthy control samples were donated by volunteers for scientific research purposes. Informed consent was obtained from all participants for the storage and research use of the biological samples. The study was carried out according to the Helsinki Declaration and was approved by the ETT-TUKEB.

Genetic studies

Genomic DNA was used for molecular genetic studies. DNA was extracted from 200 µl of venous blood treated with EDTA using the QIAamp DNA blood kit, according to the manufacturer's instructions (QIAgen, Hilden, Germany). The CYP 2C19*2 (681G-A, rs 4244285) genotype was detected using the C__25986767_70 TaqMan® Drug Metabolism Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) and fluorescent measurements were carried out with the ABI PRISM 7300 SDS machine (Applied Biosystems, Foster City, CA, USA). Individual reactions were set up using 5 µl TaqMan Universal PCR Master Mix, 0.25 µl 40x Assay Mix (C__25986767_70) and 10 ng genomic DNS and were diluted with ddH_2O-t to 10 µl volume. Thermal profile of PCR was the following: initial denaturation/activation 95°C, 10 min, then denaturation, 92°C, 15 sec then attachment and chain elongation 60°C 1.5 min, repeated through 70 cycles. Fluorescence of the samples was measured initially, during the run and after the reaction. Evaluation of fluorescence data and genotype matching was performed with the ABI PRISM 7300 SDS software.

Statistical analysis

Genotype frequencies were counted in stroke patients and healthy controls. Deviation from the Hardy-Weinberg equilibrium (HWE) and genotype association was studied with an online program (13).

Results

Frequencies of the CYP2C19*2 polymorphism (rs 4244285, 681 G>A) in ischemic stroke patients and healthy controls are described in Table II. Nine patients (2 female, 7 male) with homozygous mutation, 37 female and 38 male patients with heterozygous mutation were identified in the stroke group. Homozygous mutation was present in one female, while heterozygous mutation was found in 13 male and 40 female in the group of healthy controls.

Proportion of the minor alleles was 12.85% in the stroke group and 12.89% in healthy controls. Frequency of the minor allele was 12.87% in the total studied population. The

frequency of the major and minor alleles did not deviate significantly from the Hardy-Weinberg equilibrium neither in the stroke, nor in the control group. No significant differences were found in allele frequencies between the two groups. Frequencies of the studied SNPs in our sample do not differ from those found in other Caucasian populations.

Table II. Genotype and allelic frequencies of rs42544285 (681 G>A) polymorphism and deviation from the Hardy-Weinberg equilibrium

Groups	Genotypes			Major Allele	Minor Allele	Frequency	HWE
	GG	GA	AA	G	A	G	(χ^2)
Stroke	272 (76.84%)	73 (20.62%)	9 (2.54%)	617 (87.15%)	91 (12.85%)	0.87	2.24
Control	165 (77.46%)	55 (24.89%)	1 (0.45%)	385 (87.1%)	57 (12.9%)	0.87	2.57
Total	437 (76.00%)	128 (22.26%)	10 (1.74%)	1002 (87.13%)	148 (12.87%)	0.87	0.03

Discussion

The importance and effectiveness of clopidogrel therapy has been proven by several clinical studies (4, 7, 8, 18, 19). Effectiveness of the drug is greatly influenced by allelic variants of CYP2C19. The CYP2C19*2 variant decreases, whereas CYP2C19*17 increases activation and effectiveness of the molecule. The Food and Drug Administration (FDA) (23) made it mandatory in the USA to indicate CYP2C19*2 allele frequencies for different populations in Medication Guides highlighting the fact that effectiveness of the drug varies among patients. As clopidogrel is widely used for the prevention of cerebrovascular and cardiovascular disorders in Hungary it was important to determine the frequency of the CYP2C19*2 minor allele in the Hungarian population. The determined frequency of 12.87% is similar to the values found in other Caucasian populations and implies that it is relatively infrequent among Hungarian patients that the bioactivation of clopidogrel does not take place due to genetic factors thus increasing the risk for stroke and myocardial infarction rather than decreasing.

The fact that numerous co-medication such as proton pump inhibitors, serotonin reuptake inhibitors (SSRIs) might further decrease the effectiveness of clopidogrel even with 47 percent in case of omeprazole underscores the importance of determining the CYP2C19 allelic status.

With co-medication also heterozygous individuals might be at risk not knowing if they are able to reach a necessary level of bioactivation due to further decrease in CYP2C19 activity. When CYP2C19 inhibitors further decrease this residual enzyme function, it will not be able to activate clopidogrel to the extent of effectively inhibiting platelet aggregation. Geisler et al. (10) have found that low levels of clopidogrel response actually increase the risk of cardiovascular events in symptomatic coronary artery disease. It is now mandatory to indicate the most frequently used and most potent CYP2C19 inhibitors in the FDA drug registry (14). These are omeprazol, lansoprazol, fluconazole, ketoconazole, etravirine, felbamate, fluoxetin, fluvoxamin and ticlopidine (Table III). Among them the interaction of omeprazole and clopidogrel is the most frequent and the most studied one.

Table III. The most important drugs metabolized by the allelic variants of the CYP2C19 enzyme

Substrates	Inhibitors	Inducers
	Antibiotics	**Antibiotics**
	Chloramphenicol	Rifampicin
	Fungicides	
	Ketoconazol	
Antidepressants	**Antidepressants**	
Amitriptylin	Fluoxetin	
Clomipramin	Fluvoxamin	
Imipramin	Moclobemid	
Citalopram		
Moclobemide		
Antiepileptics	**Antiepileptics**	**Antiepileptics**
Norazepam	Felbamate	Oxcarbazepine
Diazepam	Oxcarbazepine	Topiramat
Phenytoin		
Phenobarbital		
Proton pump inhibitors	**Proton pump inhibitors**	
Omeprazol	Omeprazol	
Pantoprazol	Lansoprazol	
Antiplatelet agents		**Antiplatelet agents**
Clopidogrel		Ticlopidine
Anticoagulants		
Warfarin		

Distribution of CYP2C19*2 allele has been extensively studied (see Table I). Higher occurrences have been described in Asian populations; i.e. in the Philippines, in Japan, China and India. African, Arabic and Jewish populations have intermediate rate, while Caucasians have the lowest rate. Our results for CYP2C19*2 minor allele frequencies are in the higher range within the Caucasian population 'however' an even higher rate was published only in Croatia but that study was carried out only on a small sample of 200 individuals.

In conclusion, occurrence of the CYP2C19*2 allele in the Hungarian population is similar to other Caucasian populations, the homozygous mutation being relatively rare in cerebrovascular and cardiovascular patients. However, heterozygous mutation is present in every fifth person in the population. In these cases, interaction with CYP2C19 inhibitors such as omeprazole might further reduce the genetically impaired enzyme activity. Based on our findings, we would like to emphasize that it is important to adjust individually antiplatelet treatment in ischemic stroke patients and to take into consideration genetic factors as well as drugs taken for comorbid conditions.

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