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Short communication

Frequency of common *CYP3A5* gene variants in healthy Polish newborn infants

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Abstract:
Cytochrome P450 monooxygenases catalyze the metabolism of approximately 40-60% of widely used drugs with a A6986G *CYP3A5* polymorphism determining expresser (A6986, *1) and reduced- expresser (*3) variants with modified drug metabolism activity. In this report, the allele frequency of *CYP3A5 *1* and *3* (A6986 or G6986, respectively) was analyzed by the PCR-RFLP technique in a cohort of 200 Polish newborns from the West Pomeranian region. Of the studied group, 1% (n = 2/200) proved homozygous for the *CYP3A5*1* allele, 89% (n = 178/200) for the *3* allele, and 10% (n = 20/200) were heterozygous for *1/*3*. Similar frequencies were found in other Caucasian European populations. This study provides basic genetic data related to the metabolism of drugs, with a narrow therapeutic window in a Polish population.

Key words:
CYP3A5 variants, pharmacogenetics, drug metabolism

Introduction

The cytochrome P450 family is a group of enzymes with primary monooxygenase activity, which is involved in the metabolism of approximately 40–60% of the drugs in everyday clinical use, oxidizing a range of compounds, including steroids, fatty acids, immunosuppressants and chemotherapeutic agents (e.g., cyclosporine, trastuzumab and erlotinib). Cytochromes are transmembrane proteins bound to the endoplasmic reticulum and outer mitochondrial membrane. They are abundantly expressed in virtually every human tissue and organ, with the sole exception of striated muscle fibers and erythrocytes. The highest enzymatic activity was described for the liver and adrenal cortex.

The CYP3A subfamily (P450 3A) consists of the 3A5 enzyme and three other isoforms, namely CYP3A4, CYP3A7 and CYP3A43, as well as two pseudogenes, CYP3AP1 and CYP3AP2 [24]. Functionally, CYP3A5 is responsible for 6% to 99% of total CYP3A activity in various populations, with negligible contributions from CYP3A43 and CYP3A7 isoforms. An array of genes is responsible for the coding of various P450 isoforms, with high sequence homology within the family and the gene encoding for *CYP3A5* located on the 7q22.1 chromosome [11, 12]. Sequence data were independently published by two research groups [1, 8, 17, 20].

Gene variants of *CYP3A5* are responsible for major individual differences in enzymatic activity, strongly influencing pharmacokinetics and bioavailability of an array of drugs with a narrow therapeutic window, as well as the individual response to the drug therapy [2, 5, 15]. The most frequent single nucleotide polymorphism (SNP) of functional importance in the *CYP3A5* gene is the A to G transition in intron 3 at position 6986 (*CYP3A5*3*) [17]. This variant leads to alternative splicing with impaired protein synthesis, resulting in a decrease in metabolic activity. Conversely, the presence of the wild-type, expresser-related A6986 allele (*CYP3A5*1*) leads to the phenotype of high CYP3A5 catalytic activity. In *CYP3A5* expressers, dose requirements for certain immunosuppressants, such as tacrolimus, tend to be higher, while clearance of the anxiolytic midazolam is slower among individuals with a **3* genotype [5, 9]. It must be noted that data from various pharmacogenetic studies are often conflicting, especially in the case of midazolam. Some studies do not confirm that *CYPA5*1* allele carriers metabolize the drug better than individuals with other variants [9]. The frequency of the *CYP3A5*3* variant differs among populations and races, ranging from 27–50% in the Afro-American population, 60–70% among Asians, and up to 85-95% in individuals of Caucasian ancestry, in which the expresser variant is uncommon.

Distribution of the *CYP3A5* alleles has not been reported in the Polish population. It therefore remains unconfirmed if the allelic frequency falls within the assumed range. Moreover, as the variant affects drug metabolism of a range of drugs, including immunosuppressants (e.g., tacrolimus, cyclosporine, docetaxel, nifedipine), such exact findings can be of clinical benefit.

The aim of this study was to analyze the distribution of the *CYP3A5* variants in a group of newborn Polish children. Basic genetic data on the allele frequency in the randomly selected sample are provided, bridging the gap in the knowledge on CYP alleles in a population of Poles.

Material and Methods

Ethical issues

Local ethical committee approval was obtained for the study protocol, sample collection, DNA extraction and storage. A written formal consent for collection of umbilical blood samples, DNA extraction, storage and analysis was obtained from neonates' parents.

Samples and DNA isolation

The studied population consisted of 200 consecutively born newborns delivered at the Neonatology Department, Pomeranian Medical University, Szczecin, Poland. Gender distribution in the group was as follows: 106 female (53%) and 94 male (47%). Genomic DNA was extracted from 100 μL of infants' umbilical cord blood with the QIAamp DNA Blood Mini Kit (QIAGEN, Germany), and DNA yields were estimated by measuring the absorbance at 260 nm (A_{260}).

PCR- RFLP for *CYP3A5* variant alleles

Approximately 20 ng of genomic DNA was used in a 10 μL PCR reaction. The PCR mixture contained: the buffer (pH 8.3, 1.5 mM $MgCl_2$), 0.2 mM each of the deoxynucleotide triphosphates, 0.5 U Taq polymerase (MBI Fermentas, Lithuania) and 4 pmol each of the forward and reverse primers designed by Schaik et al. [23]. The forward primer sequence was 5'-CAT CAG TTA GTA GAC AGA TGA, and the reverse primer sequence was 5'-GGT CCA AAC AGG GAA GAA ATA (TIB MOL BIOL, Poznań, Poland). PCR reactions were performed in a Mastercycler Gradient device (Eppendorf, Germany) with the following temperature profiles: initial denaturation at 94°C for 5 min, 36 cycles of 20 s at 94°C, 40 s at 50°C and 40 s at 72°C, and a final extension step at 72°C for 7 min. Amplification was followed by digestion of a 293-bp product with the *SspI* restriction enzyme (MBI Fermentas, Lithuania) for 18 h at 37°C. The PCR digestion products were separated in 4% agarose gels, stained with ethidium bromide and recorded with the DS- 34 Polaroid Instant Camera (Polaroid, Germany) under UV light (Transiluminator 4000, Stratagene). *SspI* digestion yielded fragments of 168, 148, 125 and 20 bp (heterozygote **1/*3*), 148, 125 and 20 bp (homozygote **1/*1*), and 168 and 125 bp (homozygote **3/*3*).

Results and Discussion

The frequency of all genotypes showed no deviation from Hardy-Weinberg equilibrium (HWE). In the

Tab. 1. CYP3A5 genotypes in Polish compared with other populations

Country/(Population)	Population (n)	*CYP 3A5* allele frequency		References
		*1	*3	
Poland/(Caucasians)	Healthy newborn infants, (200)	0.060	0.940	Present study
USA/(Caucasians)	Healthy adults, (54)	0.148	0.852	[17]
German/(Caucasians)	Healthy adults, (428)	0.061	0.939	[7]
USA/(Afro-Americans)	Healthy adults, (20)	0.450	0.475	[17] *
The Netherlands/ (Caucasians)	Healthy adults, (500)	0.086	0,914	[23]
Finland/(Caucasians)	Healthy adults, (449)	0.079	0.921	[14]
Korea/(Korean)	Healthy adults, (29)	0.310	0.690	[6]
Japan/(Japanese)	Healthy adults, (200)	0.233	0.767	[10]
Tanzania/(Afro-Americans)	Healthy adults, (144)	0.510	0.190	[18] **
Sweden/(Caucasian)	Healthy adults, (136)	0.070	0.930	[18]
East Asia/(Chinese)	Healthy adults, (108)	0.245	0.755	[3]
South Asia/(Malay)	Healthy adults, (98)	0.388	0.612	[3]
South Asia/(Indian)	Healthy adults, (90)	0.406	0.594	[3]
Greece/(Greeks)	Healthy adults, (283)	0.057	0.943	[2]
North America/(Caucasians)	Healthy adults, (437)	0.093	0.907	[19]
USA/(Afro-Americans)	Healthy adults, (38)	0.658	0.342	[19]
Korea/(Koreans)	Healthy adults, (40)	0.225	0.775	[15]
Central America/(Afro-Americans)	Healthy adults, (232)	0.237	0.763	[21]
North Spain/(Spaniard)	Healthy adults, (204)	0.086	0.914	[21]
USA/(European-Americans)	Healthy adults, (23)	0.130	0.870	[9]
USA/(Afro-Americans)	Healthy adults, (34)	0.721	0.279	[9]
Finnish/(Caucasians)	Healthy adults, (754)	0.070	0.930	[22]
USA/(Afro-Americans)	Healthy adults, (25)	0.540	0.460	[11]

* Kuehl et al. [17] CYP3A5 allele frequency: *1- 0.450, *3- 0.475, *6- 0.075 ** Mirghani et al. [18] CYP3A5 allele frequency: *1-504, *3-0.194, *6-0.184, *7-0.118

studied population of 200 healthy newborn infants, 1% (n = 2) were homozygous for the **1* allele, 89% (n = 178) for the **3* allele, and 10% (n = 20) were **1/*3* heterozygotes. As was expected, among the Caucasian population, the mutated **3* allele was notably more frequent (94%) when compared to the wildtype **1* (6%). This is in accordance with previously published data, as reviewed in Table 1.

Highly similar frequencies were observed for German, Finnish, Swedish and Greek populations [2, 7, 11, 14, 17, 22]. Interestingly, the allelic frequencies described in our work are not significantly different from the ones published for the Baltic Sea area. However, the distribution of the non-expresser **3* allele is the lowest in the region [7, 14]. This confirms the trend described of the notably increasing gradient of *CYP3A5*1* allele frequency from north to south of the globe [17–19], with a smaller increase among Asians compared to individuals of Caucasian ancestry [3, 5, 10].

It should be emphasized that Poland is inhabited by a population with a high frequency of the non-expresser **3* allele, as shown in our study. These *CYP3A5* genotypes have been associated with the lower mean clearances of benzodiazepines and etopo-

side, which might increase the probability of drug-related adverse reactions, such as peripheral neuropathy [16].

Additionally, a strong association between the *CYP3A5*1* genotype and tacrolimus dose requirement was observed. This is especially important because the most effective immunosuppressive therapy is required for adequate protection against transplant rejection, in the case of chemotherapy in bone marrow recipients and lymphoproliferative disease treatment [6, 13]. This allele is infrequent in Poland and other European populations [9]. The exact correlation between *CYP3A5* genotypes and drug metabolism needs to be further analyzed, and clear relationships between genetic variants and type of CYP expression needs to be confirmed.

CYP3A5 allele frequency changes prove to be largely race- and ancestry-dependent, with high similarities described in countries from similar regions. In American populations, the observed decrease might be related to the inclusion of a population with a different ancestry into the genetic pool [19].

For this study, a random sample of newborns (all of Caucasian origin) from the northwestern area of Poland was selected. This provided basic information on the *CYP3A5* allele frequency for future pharmacogenetic studies, as well as enabling analysis of possible age-related effects on the prevalence of this variant.

Additionally, this studied neonatal population might be considered an excellent genetic sample from the largely immigrant population of Poles living in the northwestern area of the country. Random enrollment to the study with samples drawn from subsequent children delivered at the obstetric ward allowed excluding the possibility of population bias and stratification. This is possibly the only moment of life when such sampling is possible, with no division due to social differences, as all childbearing women in Poland are state insured with full and unlimited access to obstetric and gynecologic care and follow-up. Therefore, we would like to emphasize the value of this data, which may serve as a background for comparison with other population samples and could be included in case-control studies as a reference. A similarly designed study for apolipoprotein E alleles was performed by Becher et al. to provide the most adequate population allelic frequencies [4].

With the development of therapeutic options and wide access to drugs, the notion of tailored therapy will undergo further development. Therapeutic drug monitoring, currently expensive and rare in clinical use, proves most valuable in cancer and antiviral therapy. However, it might be partially replaced by genetic marker data, assuming valid research data of drug metabolism and concentrations in association with gene variants are published. It is not only desirable, but also feasible for CYP3A polymorphisms, as genotype data might allow the prediction of serum concentrations of certain drugs. Based on this data, it would be possible to adjust drug dose or select the best therapeutic options for an individual.

In summary, it might be concluded that frequency of the *CYP3A1* allele is as low as 6%. Therefore, rapid CYP3A5-dependent metabolism of immunosuppressants such as tacrolimus is infrequent in our population. Although this study was carried out in a population from a single region, the distribution is similar to the ones observed for other European populations [7, 18] and the *CYP3A5 *1* and **3* frequencies in Poland should not differ notably.

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