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SHORT COMMUNICATION

Genotype and allele frequencies of polymorphic *UGT1A9* in the Polish population

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Abstract The human UDP-glucuronosyltransferase 1A9 (UGT1A9) plays a central role in the metabolism of different therapeutic drugs, carcinogens and endobiotics. The *UGT1A9* gene shows genetic polymorphism with frequencies significantly different in populations and ethnic groups. Many of these genetic variants are directly responsible for polymorphic drug metabolism. Three crucial alleles of *UGT1A9*, *UGT1A9*3* (p.Met33Thr), **4* (p.Tyr242X), **5* (p.Asp256Asn) are associated with decrease or absence of enzyme activity, which intensify the risk of toxic effect during biotransformation. The goal of the present study was to discover frequencies of these genetic variations in 308 healthy individuals representing Polish population. The genotypes were determined by pyrosequencing. We demonstrated that the frequency of the variant *UGT1A9*3* was 0.016, which suggests the need for detailed analysis of its effect on important drugs metabolism level in Polish population. Alleles *UGT1A9*4* and *UGT1A9*5* were not present in any of the subjects. So far, no studies have been conducted in which the distribution of these alleles has been determined in the Polish population.

Keywords UDP-glucuronosyltransferase · UGT1A9 · Polymorphic drug metabolism · Polish population · Allele frequencies

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1 Introduction

UDP-glucuronosyltransferase 1A9 is a dominant isoform of UGT enzymes expressed in the liver and also in the kidney, colon, small intestine, ovary and testis (Ritter et al. 1992; Strassburg et al. 1998). Gene encoding this isoform indicates 89 % sequence identity with the pseudogene *UGT1A13p* (Gong et al. 2001). UGT1A9 plays a central role in the process of elimination of potentially toxic xenobiotics and endogenous compounds, due to its catalysis of glucuronidation of thyroid hormones, bulky phenols, steroids, fatty acids, variety of important drugs including irinotecan, flavopiridol and propofol (Watanabe et al. 2002; King et al. 2000). Polymorphic expression and variable levels of glucuronidation activity mediated by the UGT1A9 protein have been reported (Villeneuve et al. 2003; Jinno et al. 2003). The association between variable enzyme activity and existence of genetic variations in the *UGT1A9* gene and polymorphisms in the promoter region and exon 1 of this gene has been described (http://www.pharmacogenomics.pha.ulaval.ca/sgc//alleles/UGT1A/UGT1A9.htm). The most important alleles *UGT1A9*3* (p.M33T, c.98T>C), *UGT1A9*4* (p.Y242X, c.726T>G) and *UGT1A9*5* (p.D256 N c.766G>A) lead the changes of the enzyme activity. The sequence variation resulting in the substitution of methionine by threonine at codon 33 of *UGT1A9* gene (p.M33T) is associated with decreased

glucuronidation activity and is present in Caucasians with 1–3.6 % frequency (Mehlotra et al. 2007; Villeneuve et al. 2003). Also the amino acid substitution of aspartic acid to asparagine at codon 256 (p.D256N) predicts changes in phenotype, decreasing the UGT1A9 activity; however, the change at 726T>G leads to premature termination codon TAG (p.Y242X) which results in complete inactivation of protein product (reported allele frequency <1 % in Japanese) (Girard et al. 2006; Saeki et al. 2003; Jinno et al. 2003; Villeneuve et al. 2003). Allelic differences between populations and ethnic groups associated with these changes have been observed. Functional studies revealed the significance of these *UGT1A9* polymorphisms in altered metabolism and pharmacokinetics of many drugs, for example, of the immunosuppressive mycophenolate mofetil and of the anticancer drugs such as irinotecan and also of the anesthetic, propofol. These observations strongly suggest that genetic variations 98T>C, 726T>G, 766G>A of *UGT1A9* could be the main cause of the adverse effects after treatment of drugs, metabolized by UGT1A9 enzyme (Bernard and Guillemette 2004; Villeneuve et al. 2003; Jinno et al. 2003; Wolf and Potter 2004; Parke et al. 1992).

We investigated the presence of three important *UGT1A9* alleles *UGT1A9*3*, **4* and **5* in 308 subjects representing Polish population, using pyrosequencing. This study is the first demonstration of these *UGT1A9* gene polymorphisms in the population of Poland.

2 Materials and methods

2.1 Human DNA samples

Genomic DNA of 308 Polish individuals was obtained from the peripheral blood according to standard procedures using the method with guanidine isothiocyanate (GTC). Blood from the participants was collected in the collaboration with the Department of Anaesthesiology and Intensive Care Medicine, Poznan University of Medical Sciences, Poznan, Poland, and partly also derived from the Institute of Human Genetics Polish Academy of Sciences in Poznan. This study enrolled unrelated individuals, without known history of cancer or chronic diseases. Ethical approval for this study was obtained from the ethics committee of the University of Medical Sciences in Poznan, Poland.

2.2 PCR conditions and DNA pyrosequencing

The amplification of the *UGT1A9* gene fragments was performed by nested PCR using Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). Region included analyzed sequence variations 98T>C, 726T>G, 766G>A in exon 1 of *UGT1A9* gene was amplified from genomic DNA using 0.2 µM of specific primers (Korprasertthaworn et al. 2009) (Table 1). The first round PCR procedure was carried out on total volume of 25 µl containing 0.75 U of FIRE Pol® DNA Polymerase, 2.5 µl 10× buffer, 2.0 µl dNTP mix (2.5 mM each dNTP), 1.5 mM $MgCl_2$ solution and 80 ng DNA. The program started with initial denaturation at 95 °C for 2 min, followed by 25 cycles at 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 60 s. Next, 0.4 µl of the PCR products from the first step were amplified with 0.2 µM of second step primers (Table 1). The second step amplification involved 50 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s. All reagents were obtained from Solis BioDyne (Tartu, Estonia). This two-step PCR ensure the specific amplification of selected *UGT1A9* gene fragments without pseudogene *UGT1A13p* sequences (Korprasertthaworn et al. 2009). The PCR products were analyzed in 1.5 % agarose gel electrophoresis. Finally, the each second step PCR product was used as the template in pyrosequencing analysis by PSQ™ 96MA System (Qiagen) and PyroMark™ Gold Q96 Reagents (Qiagen GmbH, Hilden, Germany) as described by the manufacturer.

3 Results

The results of pyrosequencing are shown in the form of pyrograms. Figure 1 demonstrates examples of the wild type (a) as well heterozygote (b) for position 98T>C of the *UGT1A9* gene. In the heterozygous state an additional peak corresponding to cytosine is generated and signal of thymine is reduced. In our study, change in M33T was found in heterozygous state (98T/C) in 10 individuals (3.2 %). The allele *UGT1A9*3* (M33T) was present with a frequency of 0.016; however, the sequence variations 726T>G (Y242X) and 766G>A (D256N) were not found in any of the subjects (Fig. 2).

4 Discussion

Among populations genetic polymorphisms of *UGT1A9* gene are rare. However, their role in affecting glucuronidation of various drugs is clearly indicated by a number of studies (Villeneuve et al. 2003; Jinno et al. 2003; Olson et al. 2009; Takahashi et al. 2008). We decided to evaluate the distribution of these polymorphisms in the Polish population, because their clinical impact on metabolism may be the central point in the optimization of the drug treatment in the future.

Table 1 Primers used for amplification and pyrosequencing of *UGT1A9* fragments. The primers labeled with biotin are marked with asterisk

	Direction	Primer Name	Sequence	Product length (bp)
1st amplification	Forward	UGT1A9_Zf	5′-TGGTATTTCTCCCACCTACT-3′	972
	Reverse	UGT1A9_Zr	5′-CCAAAGGTGAAGTATTCTTA-3′	
2nd amplification	Forward (*)	UGT1A9_M33Tf	5′-ACCAGCCCCCTTCCTCTATG-3′	111
	Reverse	UGT1A9_M33Tr	5′-CGACCTCATGGTGAACCAGTG-3′	
	Forward	UGT1A9_Y242Xf	5′-CAAAAATGCCCTAGAAATAGCCTC-3′	81
	Reverse (*)	UGT1A9_Y242Xr	5′-CAAATTGATGTGTGGCTGTAGAG-3′	
	Forward	UGT1A9_D256Nf	5′-GCCTCTGAAATTCTCCAAACAC-3′	141
	Reverse (*)	UGT1A9_D256Nr	5′-GCAGTTGATACCACCAATGAAGAT-3′	
Pyrosequencing	Reverse	UGT1A9_M33Tseq	5′-CCAGTGGCTCCCATC-3′	
	Forward	UGT1A9_Y242Xseq	5′-AAACACCTGTTACGGAGT-3′	
	Forward	UGT1A9_D256Nseq	5′-CCACACATCAATTTGGTT-3′	

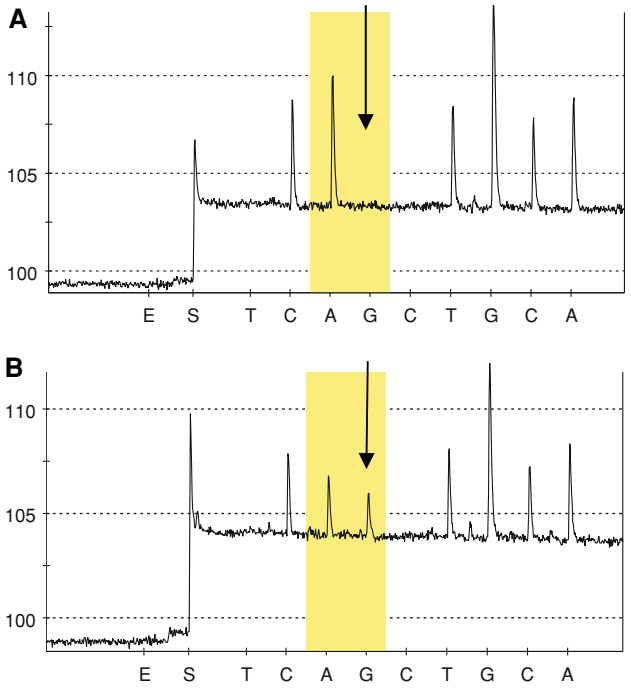

Fig. 1 Pyrograms of *UGT1A9* gene for position 98T>C (M33T). Analyzed sequence (reverse strand): CA/GTGGGCACTACCAG-TAGCTTCCCTGC. Results represent: homozygous AA wild type (**a**), and heterozygous AG (**b**)

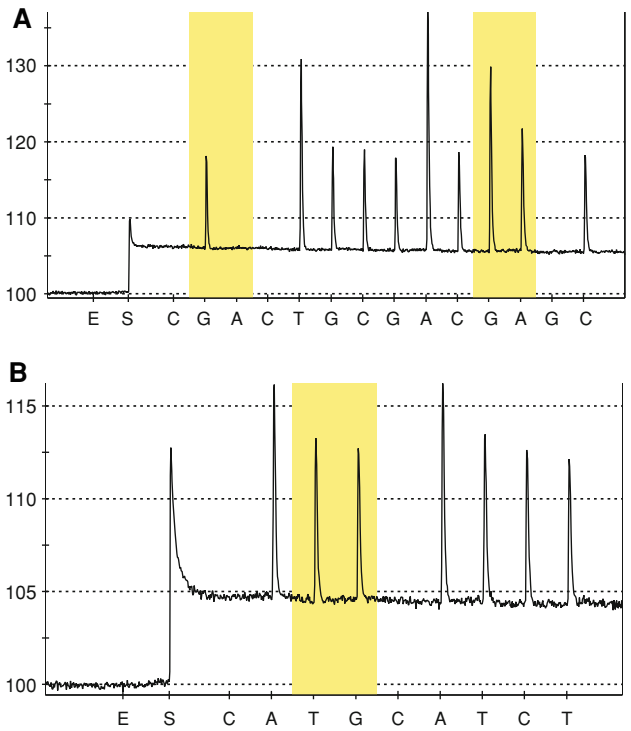

Fig. 2 Pyrograms of *UGT1A9* gene for position 766G>A (D256N) (**a**) and 726T>G (**b**). Analyzed sequences (forward strand): G/ATTGCGAACGG/AACTTTGTTTTGGACT (**a**) and AT/GGATCTCTACAGCCACACATCAATTT (**b**). Both results represent wild-type homozygous: GG (**a**) and TT (**b**)

We evaluated the presence of known genetic variants 98T>C, 726T>G, 766G>A of *UGT1A9* gene in 308 Polish individuals using pyrosequencing as a rapid genotyping method. Our study confirmed the occurrence of M33T (98T>C, *UGT1A9*3*) in Polish population with the allele frequency 0.016, which is comparable to those reported for Caucasians (0.0063–0.036) (Paoluzzi et al. 2004; Villeneuve et al. 2003) and 0.022 reported by Thibaudeau et al. (2006) (Table 2). Other studies reported the lower *UGT1A9*3* frequency, 0.01 in Caucasians-Americans, Hispanic-Americans and whites and African-Americans (Mehlotra et al. 2007; Olson et al. 2009). Villeneuve et al. (2003) observed this genetic variant in 0.03 of Caucasian-Americans, in 0.044 of French-Canadians and none in African-Americans. *UGT1A9*3* allele was not detected in Japanese population (Olson et al. 2009; Takahashi et al. 2008).

Our study demonstrates that subsequently analyzed alleles *UGT1A9*4* (Y242X) and *UGT1A9*5* (D256N) were not present in Polish individuals, in contrast to the other populations, where they have been previously described.

Table 2 Frequencies of *UGT1A9* variant alleles in Polish population compared with other populations

Population	Total number of alleles	Allele frequency in %			Reference
		*UGT1A9*3*	*UGT1A9*4*	*UGT1A9*5*	
Polish	616	1.6	0	0	Present study
Italian	327	–	1.0	1.0	Piepoli et al. (2006)
Caucasians	188	0.63	–	–	Paoluzzi et al. (2004)
White	500	1	–	–	Olson et al. (2009)
Caucasian-French-Canadian	402	2.2	–	–	Villeneuve et al. (2003)
Caucasian-American	200	3.6	–	–	
Caucasian-American	188	1	–	0	Mehlotra et al. (2007)
African-American	306	1	–	–	Olson et al. (2009)
	40	0	–	–	Villeneuve et al. (2003)
Asian-American	166	1	–	1	Mehlotra et al. (2007)
Asian	118	0	–	–	Olson et al. (2009)
Japan	200	0	0	0.5	Takahashi et al. (2008)
	602	0	0.2	0.7	Saeki et al. (2006)

– Not analyzed

Allele *UGT1A9*5* was found in Asian-Americans by Mehlotra et al. (2007) with allele frequency 0.01. Takahashi et al. (2008) demonstrated the allele frequency 0.005 in Japanese. The genetic variant D256N (*UGT1A9*5*) was also present in Italian population, with frequency 0.8 % in the pancreatic cancer patients and 1.0 % in the controls (Piepoli et al. 2006). This variant was not observed by Paoluzzi et al. (2004) in Caucasians treated with irinotecan. The Y242X substitution in the *UGT1A9*4* allele was discovered in Japanese with 0.5 % frequency (Saeki et al. 2003). Both variants Y242X and D256N have been then analyzed in Japanese using pyrosequencing with the frequencies 0.002 and 0.007 (Saeki et al. 2006). Piepoli et al. (2006) proved the existence of Y242X change in Italian control group with frequency 1.0 %; however, in the pancreatic cancers this change was not found. The results of SNPs frequency indicate allelic differences among populations (Table 2). Though both alleles were not identified in analyzed subjects, these results contribute to define population-frequencies of such important polymorphisms in Polish individuals and stimulate further studies and search for specific *UGT1A9* genotypes, significantly influencing the enzyme activity. This could be useful in reducing the risk of side effects in therapeutic treatment. Perhaps other SNPs arising from the polymorphism of this gene, located in promoter region $-118(dT)_{9>10}$ (Yamanaka et al. 2004), $-275(T>A)/-2152(C>T)$ (Lévesque et al. 2007) or intron I399C>T (Girard et al. 2006), altering the level of enzyme expression, could be prognostic factors in the Polish population.

The results of our pilot study show that the variant M33T of *UGT1A9* gene is present in 3.2 % of the Polish population and seems to be associated with decreased glucuronidation activity of this enzyme. This may suggest further analysis of the drug metabolism effect, which could be conducted for Polish individuals. Performed analysis indicate, that glucuronidation activity of M33T of *UGT1A9* variant is highly substrate-dependent. Decreased activity of M33T against 4-aminobiphenyl, but not against benzidine has been reported by Olson et al. (2009). Furthermore, 26-fold decreased activity against SN-38, irinotecan-active metabolite, was observed by Villeneuve et al. (2003). Allele *UGT1A9*3* caused reduced glucuronidation of mycophenolic acid (Bernard and Guillemette 2004), 4-hydroxyestrone and 4-hydroxyestradiol (Thibaudeau et al. 2006). Reduced activity of propofol glucuronidation has been described by Girard et al. (2004). This information suggests that genetic variant in codon 33 of *UGT1A9* may predict individual clearance rate of drugs, susceptibility of their toxicity and side effects. Further analysis in functional result of this polymorphism may afford to determination the poor-, intermediate-, extensive- and ultra rapid-metaboliser phenotype in the Polish population, which may help in optimization of the drug dose, developing personalized medicine in the future.

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