ORIGINAL ARTICLE

G2677T Polymorphism of the ABCB1 Gene in a Pakistani Population in Comparison to the Published Data on Asians and Europeans

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ABSTRACT

Background: The expression and function of P-glycoprotein (P-gp) is markedly affected by the polymorphisms in **ATP-binding Cassette member 1 (ABCB1)** gene. This transporter has many drugs as substrates and this genetic variability may be responsible for the inter-individual variability.

Objective: To investigate the frequency of G2677T- a single nucleotide polymorphism of the ABCB1 gene coding for P-gp for the first time in Pakistani population and compare it with the published data available for other populations.

Place and Duration of Study: Sampling was carried out at Combined Military Hospital, Rawalpindi from August 2012 to May 2013 and Institute of Biomedical and Genetic Engineering (IBGE).

Methodology: DNA samples were obtained from 490 subjects. The polymerase chain reaction was followed by restriction fragment length polymorphism to determine the frequencies of the genotypes. This frequency observed in our population was also compared with the published data on Asians and Europeans.

Results: The frequencies of ABCB1 G2677T genotypes were as follows: 14.9% for GG, 47.3% for GT and 37.8% for TT.

Conclusion: The homozygous TT genotype frequency is much higher in Asian populations than in European populations. In contrast, the wild-type GG genotype is more frequent in European populations than in Asian populations.

Keywords: ABCB1, frequency, genotypes, Pakistani population, P-glycoprotein, Polymerase chain reaction, polymorphism.

Introduction

A trans-membrane protein P-glycoprotein (P-gp) which functions as an efflux pump driven by energy, is pumping a variety of compounds extracellular. It is a member of adenosine triphosphate (ATP)-binding cassette transporter family and is defined as ATP-binding Cassette member 1 (ABCB1). P-gp is has 1280 amino acids with two

homologous halves which are linked by a polypeptide loop. Each half of P-gp has a trans-membrane domain and a nucleoside binding domain. The structure of P-gp is said to be necessary for transport of drug as well as it modulates protein activity and therefore determines the level of drug resistance.[1]

P-gp is mostly present in the tissues of the apical membranes of the cells facing an excretory compartment. This placement suggests that this protein will be playing a role as a pump for metabolites and drugs thus providing a protective role. Tumor cells, intestinal epithelial cells, the canalicular membrane of hepatocytes, blood-brain barrier, kidney, adrenal cortex, and placenta commonly express P-gp. P-gp is able to transport a variety of structurally dissimilar compounds. This transporter plays a substantial role in the pharmacokinetics of drugs along with secretion of steroids.[2,3,4] Therefore, any situation leading to altered activity of P-gp including functional genetic polymorphisms may alter fate of the drugs.[2]

One of the important mechanisms responsible for the resistance to anti-cancer drugs is said to be due to the overexpression of multi drug resistance proteins.[5,6] Likewise the cross-resistance to several agents is also a consequence of the transporter wide substrate specificity.[4]

P-gp is a product of the ABCB1 gene that is localized in chromosome. This gene consists of 29 exons and 2 transcription promoter sites. The sequence of nucleotides shows a number of variations. To date, 76 single nucleotide polymorphisms (SNPs) have been documented in the coding region of ABCB1. [7] G2677T is among the most commonly reported non-synonymous ABCB1 SNPs in Caucasian population. We aimed to determine the frequency of the main known variant G2677T of ABCB1 in the Pakistani population and compare it with the published data available for other populations. To our knowledge, this is the first attempt to estimate G2677T genotype frequencies in a Pakistani population.

Methodology

Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, Pakistan approved the protocol of the study. All the patients included in the study provided a written informed consent. It comprised of 490 adults both males and females randomly selected through non-probability consecutive sampling, belonging to different regions of Pakistan to provide representation from all areas. Subjects having any history of chronic diseases like cancer, hepatitis, human immunodeficiency virus infection, cardiac and neural diseases or those receiving continuous medical treatment (substrates for P-gp) were excluded from the study. The genetic analysis was carried out at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. A blood sample amounting to 5 ml was taken from all the patients.

DNA was extracted from whole blood in accordance with the organic methods.[8] Polymerase chain reaction (PCR) was followed by restriction fragment length polymorphism (RFLP) was used for genotyping of G2677T. The genomic DNA was amplified using forward and reverse primer pairs. The PCR was carried out in a final volume of 20 μl which contained 10X PCR buffer without Mg^{2+}, 25 mM $MgCl_2$, 2 mM dNTPs, 5U Taq polymerase, 10 μM forward and reverse primers and 40*g genomic DNA. *Ban1*a restriction enzyme was used to digest the products of PCR. The homozygous individuals with major allele produced a single fragment of 224 bp. The heterozygous individuals produced three fragments of 224 bp, 198 bp and 26 bp. The homozygous individuals with minor allele produced 198 bp and 26 bp fragments. The 26 bp fragment was not visible on agarose gel.

Statistical Analysis: Through direct counting, the frequencies of the genotype were calculated. The comparison of genotype frequencies with the data for other populations was done through chi square test. A p value of less than 0.05 was considered significant.

Results

Image 1 depicts the results of G2677T genotyping.

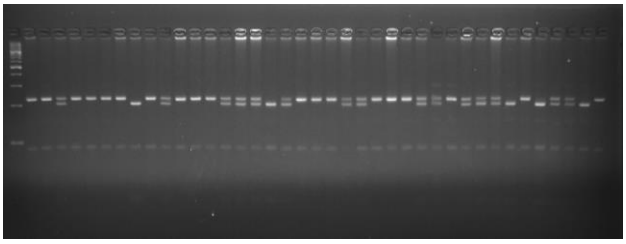

Image. 1. Electrophoresis patterns as analyzed by PCR-RFLP. Homozygous wild type GG (224bp); heterozygous GT (224bp,198bp, 26bp) and homozygous variant type TT (198bp, 26 bp).

73 patients had GG genotype, 232 patients had GT and 185 patients had TT genotype. The frequencies of ABCB1 2677 genotypes in our study were as follows: 14.9% for GG, 47.3% for GT and 37.8% for TT.

Discussion

In this study we evaluated frequencies of the most common polymorphism G2677T in the Pakistani population. This was also the first genetic polymorphism that was identified in the ABCB1 gene.[9] Later in 2000 Hoffmeyer and colleagues revealed the importance of heritable mutations in ABCB1 affecting drug disposition. In their study 15 naturally occurring SNPs were identified in healthy Caucasians.[10] Their study initiated rigorous debates, as the subsequent association studies on these polymorphisms showed marked inconsistency. Because ABCB1 polymorphisms does effect the pharmacokinetics and pharmacodynamics of drug substrates and thus produce an impact on the consequences of certain diseases[11], the conclusion that the therapeutic approach can be individualized on the basis of ABCB1 polymorphism can be drawn. Throughout the world remarkable variations in genotype frequencies of G2677T have been observed however we had no data for the Pakistani population. This aspect will help in understanding variability in drug response from this region of the world.

A comparison between the genotype frequency distribution of G2677T SNP in the Pakistani population and other populations from previous studies is summarized in Table I. The frequencies of genotypes of G2677 polymorphism of ABCB1 vary among the different population. The observed frequency of homozygous TT polymorphism in this study at this site (0.38) is very similar to that found in the Indian[12] (0.41) population. The frequency of the 2677 TT genotype determined in the present study is significantly higher than that reported in the Serbian[13] (0.15), German[14](0.16), Russian[15] (0.18), Portuguese[16] (0.26), Polish[17](0.17), Czech[18] (0.22), Japanese[19] (0.18) and Chinese[20] (0.21) populations. This study showed that the frequency distributions of heterozygous GT variant were similar between the Pakistani (0.47), Serbian[13] (0.52), German[14] (0.49), Russian[15] (0.45), Portuguese[16] (0.43), Polish[17] (0.40) and Czech[18] (0.47) populations. The frequency of this genotype was statistically different from Japanese[19] (0.32), Chinese[20] (0.37) and Indian[12] (0.31) populations. The homozygous GG genotype frequency detected in our study (0.15) is much closer to that in the Indians[12] (0.14), Japanese[19] (0.19) and Chinese[20] (0.18) population. In contrast, the incidence of CC genotype in the Pakistani population is statistically lower than that observed in the Serbians[13] (0.26), German[14] (0.31), Russian[15] (0.30), Portuguese[16] (0.31), Polish[17](0.39) and Czech[18] (0.30). As can be seen in Table I, the homozygous TT genotype frequency is much higher in Asian populations than in European populations. In contrast, the wild-type GG genotype is more frequent in European populations than in Asian populations.

Table I. Comparison of G2677T of Pakistani population with other populations

Countries	G2677T		
	GG	GT	TT
Pakistani	0.15	0.47	0.38
Serbian (n = 158)	0.26*	0.52	0.15*
German (n = 461)	0.31*	0.49	0.16*
Russian (n = 290)	0.30*	0.45	0.18*
Portuguese (n = 100)	0.31*	0.43	0.26*
Polish (n = 204)	0.39*	0.40	0.17*
Czech (n = 189)	0.30*	0.47	0.22*
Japanese (n = 154)	0.19	0.32*	0.18*
Chinese (n = 200)	0.18	0.37*	0.21*
Indian (n = 87)	0.14	0.31*	0.41

*$p<0.05$

Conclusion

In conclusion, this is to the best of our knowledge the first major study reporting the ABCB1 G2677T genotype distribution in a Pakistani population and findings suggest a significant difference in the frequency compared to other populations. This difference may be responsible for the differences in the response to different drugs in these populations. This can help in designing further studies with this polymorphic variant in our population increasing the validity of the studies. It will be of prime importance to the drugs with low therapeutic window. This will help to prevent the development of serious adverse drug reactions. In the future perspectives, routine testing for the SNPs can become a useful tool for optimizing the dosing and therapy of the drugs that are substrates for P-gp.

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