FREQUENCY OF COMMON ABCB1 GENE VARIANT C3435T POLYMORPHISM IN PAKISTANI POPULATION

Kulsoom Farhat, Akbar Waheed, Nusrat Nazir, Muhammad Ismail*, Qaisar Mansoor*, Anwar Kamal Pasha**
Army Medical College, National University of Sciences & Technology (NUST) Islamabad, *Institute of Biomedical & Genetic Engineering Islamabad, **Armed Forces Institute of Dentistry

ABSTRACT

Objective: To determine the frequency of the C3435T; a single nucleotide polymorphism of the ABCB1 gene for the first time in Pakistani population and compare it with the data available from other populations.

Study Design: A cross-sectional study.

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).

Material and Methods: The genotype frequency of C3435T polymorphism of ABCB1 gene was investigated in 491 Pakistani subjects. This frequency observed in our population was also compared with the published data on Asians and Caucasians.

Results: The distribution of frequencies of C3435T genotypes in our study population were; CC 7.1%, CT 59.1% and TT 33.8%. The Pakistanis differed significantly from Asian populations in the distribution of the TT genotype of C3435T ABCB1 ($p<0.05$). The study population also differ significantly in the distribution of CC genotype from rest of the populations compared ($p<0.05$).The data obtained may give the basis for predicting effects of drugs that are substrates for ABCB1 in Pakistani population and may be useful for individualized therapy of some diseases.

Keywords: ABCB1, Genotypes, Pakistani population, P-glycoprotein, Polymerase chain reaction, Polymorphism.

INTRODUCTION

A trans-membrane transporter-P-glycoprotein (P-gp) plays an important role in the efflux of drugs and thus influences the drug treatment outcome. Primarily being over expressed in tumor cells, it was found to be associated with resistance to chemotherapeutic agents[1]. Consequently, the localization of P-gp was identified at many sites in the body and it included blood-brain barrier, liver, intestine, kidneys, placenta, testes, salivary glands and other blood tissue barriers. Moreover P-gp has also been found in stem cells, mononuclear cells and macrophages, suggesting its physiological function over there too[2]. P-gp transports a wide range of substrates and thus plays a significant role in bioavailability, distribution and excretion of many drugs[3]. A difference in expression and function of P-gp has been identified responsible for inter-individual differences, and one reason for this difference is said to the genetic variability in the form of polymorphism. Up till now over 60 single nucleotide polymorphisms (SNPs) and 3 insertion/deletion polymorphisms naturally occurring in different populations have been reported in ABCB1[4].

In most of the studies done so far the functional impact of these SNPs on the P-gp remains unclear however some among these do have proved their functional relevance. And to be more specific, the C3435T- a silent but simultaneously a functional polymorphism has been studied repeatedly in different populations and various disease conditions[5-8].

The frequencies of the homozygous and heterozygous genotypes of C3435T have been studied in various populations including Caucasians and Asians where a large difference has been revealed among these populations. We in our study have analyzed an existing silent but functional ABCB1 C3435T polymorphism in a Pakistani population.This is the first ever study from this area to estimate C3435T genotype frequencies in a Pakistani population.These frequencies were then also compared with those in other populations.

Correspondence: Dr Kulsoom Farhat, Dept of Pharmacology, Army Medical College, Rawalpindi, Pakistan
Email: kulsoompasha@yahoo.com
Received: 26 Mar 2014; Accepted: 19 May 2014

MATERIAL AND METHODS

It was a cross-sectional study that was conducted at Combined Military Hospital, Rawalpindi from August 2012 to May 2013. analysis was performed in accordance with the principles of the Declaration of Helsinki.

The genomic DNA from whole blood was isolated using the standard organic methods[9].

Table-1: Comparison of C3435T of Pakistani population with other populations.

Countries	C3435T			References
	CC	CT	TT	
Pakistani (n= 491)	0.07	0.59	0.34	(This study)
Serbian (n = 158)	0.19*	0.54	0.27	Milojkovic *et al*, 2011
German (n = 461)	0.21*	0.50*	0.29	Cascorbi *et al*, 2001
Russian (n = 290)	0.21*	0.49*	0.30	Gaikovitch *et al*, 2003
Portuguese (n = 100)	0.12	0.47*	0.41	Cavaco *et al*, 2003
Turkish (n = 150)	0.20*	0.53	0.27	Gumus-Akay *et al*, 2008
Polish (n = 204)	0.22*	0.51	0.27	Kurzawski *et al*, 2006
Czech (n = 189)	0.21*	0.45*	0.34	Pechandova *et al*, 2006
UK (n = 190)	0.24*	0.48*	0.28	Ameyaw *et al*, 2001
Spanish (n = 204)	0.27*	0.51	0.22*	Vicente *et al*, 2008
Japanese (n = 154)	0.36*	0.47*	0.17*	Komoto *et al*, 2006
Chinese (n = 200)	0.30*	0.53	0.17*	Zhang *et al*, 2008
Indian (n = 87)	0.18*	0.37*	0.45*	(Chowbay *et al*, 2003)

* $p<0.05$ compared with the Pakistani population.

Unrelated healthy volunteers both males (n=220) and females (n=271)between the ages of 18 to 65 years were included in the study through non-probability consecutive sampling. Subjects having any history of chronic diseases like cancer, hepatitis, HIV, cardiac and neural diseases or those receiving continuous medical treatment (substrates for P-gp) were excluded from the study. Consent was taken from all the subjects in writing. All the subjects were recruited in a way that they provided representation from all regions of Pakistan including Punjab, Sindh, Balochistan, Khyber Pakhtunkhwa, Hazara/Baltistan and Azad Jammu and Kashmir. This study was approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, Pakistan.A blood sample measuring to 5 ml was taken from all the subjects included in the study.The analytical procedures were carried out at Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. The genetic

The genotyping for C3435T was made by PCR-RFLP. The amplification of DNA was carried out using forward and reverse primers for the region harboring the C3435T. The forward primers were: 5'- TGC AGG CTA TAG GTT CCA GG – 3' and the reverse primers were: R5'- TTT AGT TTG ACT CAC CTT CCC G – 3'. The PCR was then carried out. The digestion of PCR products were carried out with *Mbo1* restriction enzyme[10]. There were three fragments of 172 bp, 60 bp,16bpin individuals homozygous for major allele C. There were four fragments of 248bp, 172 bp, 60 bp, 16b pin individuals heterozygous for both the major and minor allele T and C. There was a single fragment of 248 bp in individuals homozygous for minor allele T (Fig-1).

Microsoft excel was used for data analysis. Mean and standard deviation were calculated for age. Frequency and percentage were calculated for gender and genotype. Allele and genotype frequencies were calculated by direct counting. Chi square test was used to compare the observed genotype frequencies to

published data for other populations. A *p* value of less than 0.05 was considered significant.

RESULTS

Genotyping of C3435T SNP of ABCB1 gene was assessed by PCR-RFLP in 491 Pakistani subjects with an average age of 42.67 years (SD=8.74). Two hundred and seventy one (55.2%) were females and 220 were males (44.8%). Figure 1 was the genotype results for ABCB1 C3435T polymorphisms. After digestion with *MboI* restriction enzyme the genotypes CC, CT and TT was classified into different band sizes.

Out of 491 subjects, 35 (7.1%) had CC genotype, 290 (59.1%) had CT and 166 (33.8%) had TT genotype. The comparison of C3435T of Pakistani population with other populations is given in Table-1 and Fig-2.

DISCUSSION

How one responds to the drug is said to be largely influenced by the genetic makeup and it is here that the role of polymorphism in human genome arises. These polymorphisms can alter the pharmacokinetics as well as dynamics of the drugs and thus can modify the response of the individuals to the drugs. The pharmacogenetic testing in this way can be quite useful in adjusting the therapy with the drugs that are the substrates of P-gp which is encoded by ABCB1. This dose adjustment will in turn increase the efficacy and safety of drugs.

Table-1 shows the comparison between the distribution of frequencies of CC, CT and TT genotypes in the Pakistani population and other populations; data being retrieved from previous studies. The frequencies of genotypes of C3435T polymorphism of ABCB1 vary among different population. The frequency of TT polymorphism reported in this study (0.34) is close to that found in the Serbian[11] (0.27), German[12] (0.29), Russian[13] (0.30), Portuguese[14] (0.41), Polish[15] (0.27), Czech[16] (0.34) and Caucasians of UK[17] (0.28). The frequency distribution of TT genotype of 3435 in the present study is significantly different from that documented in the Spanish[18] (0.22), Japanese[19] (0.17), Chinese[20] (0.17) and Indian[21] (0.45) populations ($p<0.05$). This study has depicted that the frequency distributions of CT heterozygous variant were similar between the Pakistani (0.59), Serbian[11] (0.54), Turkish[22] (0.53), Polish[15] (0.51), Spanish[18] (0.51) and Chinese[20] (0.53) populations. The frequency of this genotype was statistically different from German[12] (0.50), Russian[13] (0.49), Portuguese[14] (0.47), Czech[16] (0.45), Caucasians of UK[17] (0.48),

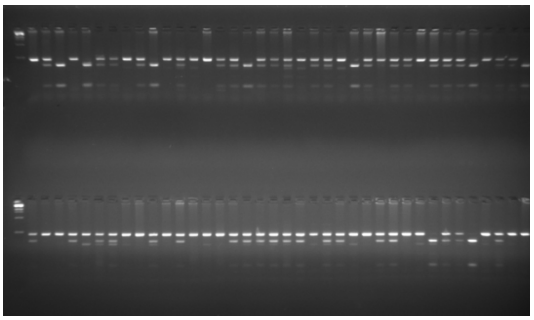

Figure-1: Electrophoresis pattern for ABCB1 C3435T alleles analyzed by PCR-RFLP. Homozygous wild type CC (172bp, 60 bp, 16 bp), heterozygous CT (248 bp, 172 bp, 60 bp,16 bp). homozygous variant type TT (248 bp).

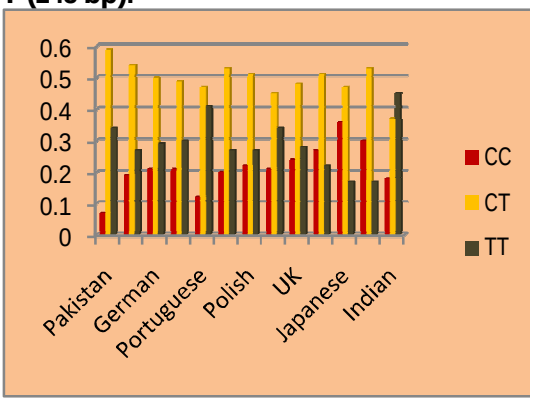

Figure-2: Comparison of C3435T of Pakistani population with other populations.

Japanese[19] (0.47) and Indian[21] (0.37) populations ($p<0.05$). We (0.07) are much closer to Portuguese[14] (0.12) in the distribution of the frequency of homozygous CC genotype. However this frequency in our population is significantly different from that reported in Serbian[11] (0.19), German[12] (0.21), Russian[13] (0.21), Portuguese[14] (0.12), Turkish[22] (0.20), Polish[15] (0.22), Czech[16] (0.21), Caucasians of UK[17] (0.24), Spanish[18] (0.27).Japanese[19] (0.36),

Chinese20 (0.30) and Indian[21] (0.18) population (p<0.05). As can be seen in Table-1, the frequency of TT genotype in comparison to Asian populations is statistically different. The wild-type CC genotype is significantly different from the rest of the populations compared.

To conclude we have put forward the frequency distribution of C3435Tgenotypes in a Pakistani population. We have observed significant difference in the genotype frequency compared to other populations. This study has provided a framework for future pharmacogenetic and pharmacokinetic studies on this polymorphic variant of ABCB1 gene in the Pakistani population. This will promote validation of more and more studies besides decreasing the economic costs and ethical risks for the individuals included in such studies. This knowledge of high frequency of functional polymorphisms in our population is also of prime importance to the practicing physicians, more specifically when they are prescribing drugs having a narrow therapeutic window. They will thus prevent the development of therapeutic failure and serious adverse drug reactions. We can foresee that in the near future this routine testing for the SNPs will become a useful tool-adjusting the dose of the drugs that are substrates for P-gp.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Gottesman MM. Mechanisms of cancer drug resistance.Annu.Rev.Med 2003; 53: 615-627.
2. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin. PharmacolTher2004; 75: 13-33.
3. Ishikawa T, Hirano H, Onishi Y, Sakurai A, Tarui S. Functional evaluation of ABCB1 (P-glycoprotein) polymorphisms: high-speed screening and structure-activity relationship analyses. Drug. MetabPharmacokinet 2004; 19 : 1-14.
4. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. Oncogene 2003; 22: 7468-7485.
5. Kroetz DL, Pauli-Magnus C, Hodges LM, Huang CC, Kawamoto M, Johns SJ, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 2003; 13: 481-494.
6. Jamroziak K, MIynarski W, Balcerczak E, Mistygacz M,Trelinska J, Mirowski M, et al. Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukaemia.Eur J Haematol2004; 72, 314-321.
7. Xhemo E, Fajac A, Boire JY, Levy P. MDRView: a visualization of the polymorphisms of MDR1(ABCB1) gene in breast cancer. ConfProc IEEE Eng Med Biol. Soc2007; 4592-4594.
8. Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. ProgNeuropsychopharmacolBiol Psychiatry2008; 32:398–404.
9. Sambrook J, Fritich EF, Maniatis T. 1989. Molecular cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, USA.
10. Sipeky C, Csongei V, Jaromi L, Safrany E, Maasz A, Takacs A, et al. Genetic variability and Haplotype profile of MDR1 in Roma and Hungarian population samples with a review of literature. Drug Metab Pharmacokinet 2011; 26(2): 206-215.
11. Milojkovic M, Stojnev S, Jovanovic I, Ljubisavljevic S, Stefanovic V, Sunder-Plassman R. Frequency of the C1236T, G2677T/A and C3435TMDR1gene polymorphisms in the Serbian population.Pharmacol Rep 2011; 63: 808-814.
12. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, , et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1gene in white subjects. Clin. Pharmacol. Ther 2001; 69: 169-174.
13. Gaikovitch EA, Cascorbi I, Mrozikiewicz PM, Brockmoller J, Frotschl R, Kopke K, et al: Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. EurJ ClinPharmacol 2003; 59: 303–312.
14. Cavaco I, Gil JP, Gil-Berglund E, Ribeiro V: CYP3A4 and MDR1 alleles in a Portuguese population. ClinChem Lab Med 2003; 41: 1345-1350.
15. Kurzawski M, Pawlik A, Górnik W, DroŸdzik M: Frequency of commonMDR1gene variants in a Polish population. Pharmacol Rep 2006; 58: 35–40.
16. Pechandova K, Buzkova H, Slanar O, Perlik F. Polymorphisms of the MDR1Gene in the Czech Population. Folia Biologica 2006; 52: 184-189.
17. Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A et al. MDR1pharmacogenetics: frequency of the C3435T mutation in exon 26 significantly influenced by ethnicity. Pharmacogenetics 2001; 11: 217–221.
18. Vicente J, Sinues B, Fanlo A, Vasquez P, Medina J , Martinez-Jarreta B. Polymorphisms of the MDR1gene in Central Americans and Spaniards. MolBiol Rep 2008; 35: 473–478
19. Komoto C, Nakamura T, Sakaeda T, Kroetz DL, Yamada T, Omatsu H, et al. MDR1haplotype frequencies in Japanese and Caucasian, and in Japanese patients with colorectal and esophageal cancer. Drug Metab Pharmacokinet 2006; 21: 126–132.
20. Zhang Y, Jiang XH, Hu YQ, Li ZR, Su L, Wang ZG, et al. MDR1 genotypes do not influence the absorption of a single oral dose of 600 mg valacyclovir in healthy Chinese Han ethnic males. Br J ClinPharmacol 2008; 66: 247–254.
21. Chowbay B, Cumaraswamy S, Cheung Y B, Zhou Q, Lee E J. Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 hap-lotypes on cyclosporin disposition in heart transplant recipients. Pharmacogenetics 2003; 13: 89-95.
22. Gumus-Akay G, Rustemoglu A, Karadag A andSungurogluA. Genotype and allele frequencies of MDR1gene C1236T polymorphism in a Turkish population. GenetMol Res 2008; 7: 1193–1199.