

Evaluation of G2677T/A polymorphism of *MDR1* gene by polymerase chain reaction in Mazandaran province, Iran

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Abstract

The human *MDR1* gene encodes for a P-glycoprotein (PGP), which acts as an efflux pump that transports a large variety of substrates from the inside of cells to the outside until protection against xenobiotics. The G2677T/A polymorphism in exon 21 is associated with PGP expression and function in humans. The present study was aimed to determine the frequencies of this polymorphism in a healthy population from Mazandaran province of Iran. A total of 120 unrelated healthy subjects from Mazandaran province, residing in Sari, coming for blood donating at Sari Blood Transfusion Center were enrolled. Genomic DNA was extracted from the peripheral blood lymphocytes of each subject. All subjects were genotyped for G2677T/A polymorphism by polymerase chain reaction-restriction fragment length polymorphism method.

The genotype frequencies were G2677G (65%), G2677T (20.83%), G2677A (14.17%) and TT, AA, TA genotypes were not observed. Moreover, frequency of G allele (82.5%) was significantly ($p < 0.05$) higher than the T (10.42%) and A (7.08%). This is the first study to investigate the G2677T/A polymorphism of *MDR1* gene in population from Mazandaran province of Iran. These data may be relevant for dose recommendation of PGP substrate drugs and can help for individualizing drug therapy of organ transplantation and important diseases such as cancer and AIDS, congestive heart failure and etc.

Keywords: P-glycoprotein, G2677T/A polymorphism, Iranian population, *MDR1*

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Introduction

ATP Binding Cassette (ABC) transporters are a family of transmembrane proteins that translocate molecules across biological membranes (1). Human Multidrug Resistance 1 (*MDR1*, also referred to as ABCB1) is probably the best characterized of the ABC xenobiotic efflux transporters that is located on chromosome 7q21 (1,2). The *MDR1* gene encodes P-glycoprotein which functions as an ATP-dependent exporter of substances from inside of cells to the outside (3). PGP was first observed in

tumor cells for conferring resistance against anti-cancer agents (4,5). In addition, it is expressed in normal tissues including White blood cells, liver, kidneys, small and large intestine, the biliary ducts, pancreas, placenta, brain and testis (6).

PGP is involved in the absorption, distribution and excretion of xenobiotics, numerous drugs and toxins into bile and urine (7-9). PGP can protect the organism from environmental toxins and carcinogens, it can also prevent the penetration of drugs

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into the brain, testis or fetus because of its accumulation in the blood-tissue barrier and blood-fetus barrier (10-13). PGP transports broad substrates including anti-HIV drugs, cardiac glycosides, steroids, several anticancer drugs, antibiotics, immunosuppressants and drugs in other categories (14).

Recently several single nucleotide polymorphisms (SNPs) have been reported in the *MDR1* gene (15). Polymorphisms in this gene can affect the metabolism of drugs, the pharmacological action and toxicity profile of a vast number of therapeutic agents (16,17). The first mutations in *MDR1* gene in normal cells were G2677T/A, C3435T and G2995A that were defined by Mickley (18). Among them, G2677T/A was the most extensively evaluated in relation to PGP expression.

G2677T/A is a non-synonymous SNP, which is located at exon 21 in the second transmembrane domain. G2677T mutation leading to the substitution of Serine in place of the usual Alanine at amino acid 893 and G2677A mutation leading to amino acid exchange from Alanine to Threonine (13). G2677T/A polymorphism has impact on the expression and activity of PGP and alters in vivo drug disposition and drug effects (19-21).

So far, significant interethnic differences in allele and genotype frequencies of C3435T and other *MDR1* SNPs have been reported. Considering the well-known influence of *MDR1* on the bioavailability and pharmacokinetics of various drugs, genotyping of *MDR1* polymorphisms and determination of haplotypes may become an important tool for predicting individual susceptibility to development of drug resistance (15). In this investigation, allele and genotype frequencies of the G2677T/A *MDR1* gene was determined in a population from Mazandaran province (Sari, Iran) to obtain data relevant for this ethnic group.

Materials and methods

Study population

The present study included 120 randomly selected (110 men and 10 women; mean age 35.78 ± 10.08), healthy, unrelated individuals from Mazandaran province, residing in Sari, coming for blood donating at Sari Blood Transfusion Center during the period of May 2012– July 2012 fulfilling our inclusion and exclusion criteria. Individuals were eligible for study if they had no history of any chronic diseases or cancers and were enrolled after a written informed consent was obtained. Ethnicity was recorded by self-report. The protocol of the study was approved by the research ethics committee of Mazandaran University of Medical Sciences.

PCR Amplification

DNA was isolated from whole blood samples using a DNGTM plus Kit (Cinnagene, Iran) according to the manufacture's protocol. G2677T/A polymorphism was detected based on Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primers for G2677T/A polymorphism in exon 21 were:

Forward : 5'-TACCCATCATTGCAATAGCAG-3'

Reverse (G2677G) : 5'-TTTAGTTTGACTCACCTTGCTAG-3'

Reverse (G2677T) : 5'-TTTAGTTTGACTCACCTTTCTAG-3'

Reverse (G2677A) : 5'-TTTAGTTTGACTCACCTTCCC-3'

The PCRs were performed in a 30 μ L reaction volume containing 2 μ L of DNA, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 1U Taq DNA polymerase, 3 μ L of 10x PCR Buffer, 1 μ L of each forward and reverse primers and 21 μ L water.

The PCR protocol was as follows: initial 5 min at 94 °C followed by 38 cycles, consisting of denaturation for 40s at 94 °C,

annealing for 40s at 60 °C to 62 °C, and extension for 40s at 72 °C and terminal elongation was performed for 5min at 72 °C. Amplified segments electrophoresed on 1% agarose gel containing 5 µg/ml ethidium bromide (22) .

Detection of G2677T/A polymorphism

The genotype frequencies for each SNP in the study were determined by the RFLP method. The GenBank accession number of MDR1 reference used in this study is M14758. The PCR product (107 bp) was digested at 37 °C for 16 h with 2 U of NheI restriction enzyme for G2677G, 2 U of XbaI restriction enzyme for G2677T and 2 U of AfaI restriction enzyme for G2677A (22). The restriction digested products were analyzed by electrophoresis on 3% agarose gel containing ethidium bromide and visualized under UV illumination.

Digestion fragments for G2677T/A polymorphism are shown in Table 1

Sequencing

DNA sequencing method was carried out for several PCR product.

Statistical analysis

Genotype and allele frequencies between groups were analyzed using SPSS version 18 included the chi-square test (χ^2). A p-value of less than 0.05 was considered

statistically significant. Ninety-five percent confidence intervals were calculated for all observed allele and genotype frequencies. Allele and genotype frequencies for SNPs were assessed for deviation from the Hardy-Weinberg equilibrium.

Results

Demographics of the study groups

We analyzed samples obtained from 120 healthy unrelated individuals to detect G2677T/A polymorphism of the MDR1 gene in 2677 position.

Allele frequency and genotype distribution

Fig.1 illustrates the results of the genotypes by their PCR product length. The allele and genotype frequencies of MDR1 variants in 2677 position are given in Table 2. Eight PCR products spanning MDR1 G2677T/A were sequenced and the results were consistent with those determined by the PCR. Results for two representative samples carrying 2677 GG and GT are shown in Fig.2.

In our study, the frequencies of the G, T and A alleles was obtained 82.5%,

Table 1 Digestion fragments for G2677T/A polymorphism (22)

	Length (bp)	Restriction Enzyme	Cutting Position	Wild/Wild	Wild/Mutant	Mutant/Mutant
G2677G	107	NheI	83	24,83	24,83,107	107
G2677T	107	XbaI	83	107	24,83,107	24,83
G2677A	107	AfaI	83	107	24,83,107	24,83

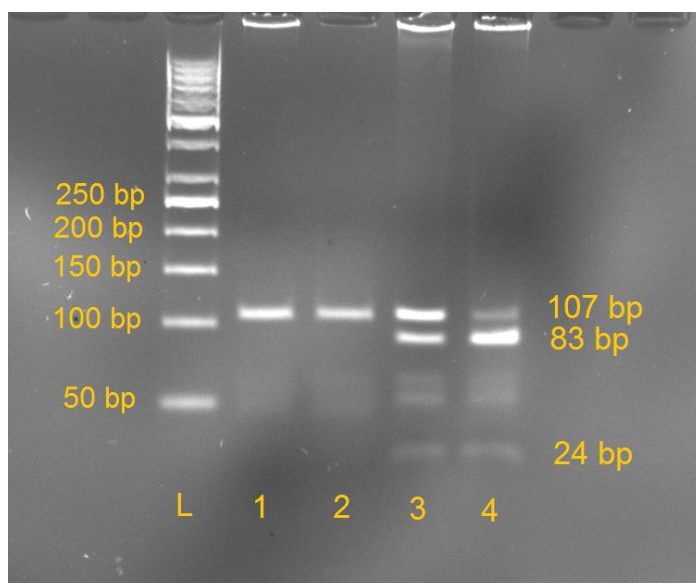


Figure 1 Results of PCR-RFLP analysis of MDR1 G2677T/A SNP. L: 50 bp DNA ladder ; Lane 1: PCR product (107 bp) ; Lane 2 : GG genotype ; Lane 3 : GT genotype ; Lane 4 : GA genotype.

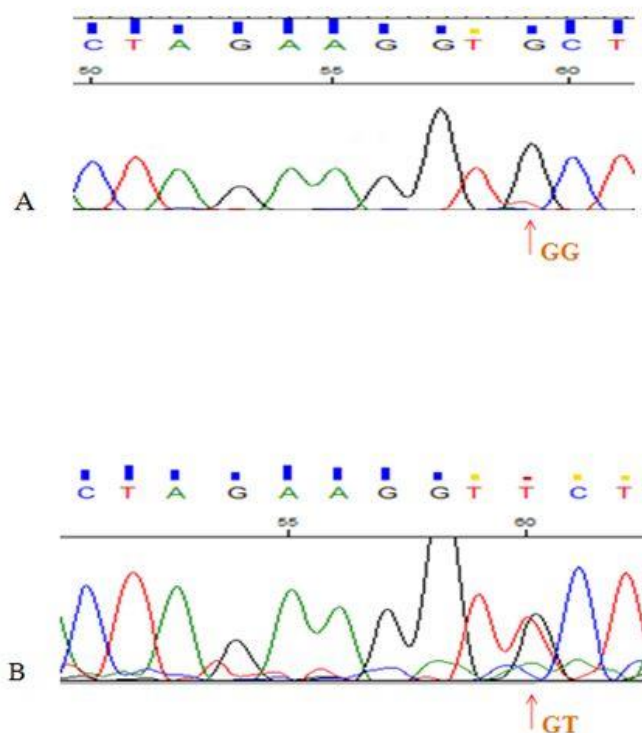


Figure 2 DNA sequences of MDR1 G2677T/A single nucleotide polymorphism. A : GG genotype , B GT genotype.

Table 2 The allele and genotype distributions of *MDR1* G2677T/A in Mazandaran province (Sari)

	Frequency (%)	95% CI
MDR1 G2677T/A genotype*		
GG	78 (65)	73.53 – 56.46
GT	25 (20.83)	28.09 – 13.56
GA	17 (14.17)	20.40 – 7.93
MDR1 G2677T/A allele*		
G	198 (82.5)	87.30 – 77.69
T	25 (10.42)	14.28 – 6.55
A	17 (7.08)	10.32 – 3.83

*P < 0.05.

CI: confidence interval.

10.42% and 7.08% respectively (P < 0.05). GG, GT and GA genotype frequencies were 65, 20.83 and 14.17% respectively (P < 0.05) and TT, AA, TA genotypes were not observed. The observed genotype frequencies did not deviate significantly from those expected at Hardy-Weinberg equilibrium.

We found that frequencies of *MDR1* G allele in our population is significantly higher than that the usual rate in Asian (23-26), Caucasian (15,27-32) and American (33) populations, but the frequencies of *MDR1* T allele is significantly lower than all studied-populations. Moreover, frequencies of *MDR1* A allele in our population is similar to Indian (26), higher than Caucasian (15, 27-32) and American (33) populations, lower than Chinese (25), Japanese (24) and Korean (23) populations. We have shown the allele and genotype frequencies of the G2677T/A polymorphism in

Mazandaran province (our study) and other populations in Table 3 and Table 4.

Discussion

Genetic polymorphisms have important effects on the response of a patient to drug therapy, on drug metabolizing enzymes and on the target of drugs (34-37). Expression of PGP, the product of the *MDR1* gene, is an important factor influencing the bioavailability of many cardiovascular and anticancer medications with a narrow therapeutic window. Because *MDR1* polymorphisms have an impact on the pharmacokinetic and pharmacodynamic profiles of drug substrates and directly influence the outcome and prognosis of certain diseases, it is clear that *MDR1* polymorphism analysis can provide important information to optimize the individualized therapeutic approach (15). The Present study investigated the frequency of a commonly known *MDR1*

Table 3 Comparison of allele frequencies of MDR1 G2677T/A polymorphism reported for Iranian population with other populations published.

Population	Allele frequency				Reference	
	n	G	T	A		
Asian	Iranian (Mazandaran province)	120	0.825	0.104	0.070	This study
	Indian	87	0.333	0.598	0.069	(26)
	Chinese	200	0.417	0.450	0.133	(25)
	Japanese	154	0.428	0.406	0.166	(24)
	Korean	632	0.438	0.391	0.171	(23)
Caucasian	British	285	0.579	0.396	0.025	(32)
	Polish	204	0.595	0.385	0.020	(31)
	Serbian	158	0.530	0.430	0.040	(15)
	Czech	189	0.539	0.458	0.003	(30)
	Bulgarian	160	0.559	0.441	N.A	(29)
	Scottish	370	0.512	0.498	N.A	(28)
	Slovenian	355	0.600	0.400	N.A	(27)
American	Brazil	106	0.610	0.380	0.010	(33)

G: wild- G: G: wild-type allele, T,A: mutant allele, n: number of subjects.

genetic polymorphism, G2677T/A, in a sample of Iranian population (Mazandaran province, Sari). As indicated in Table 3, the GG genotype frequency in our population was found 82.5% that is higher than Asian, Caucasian and American populations. The frequency of GT genotype in our population is lower than all populations. Our results showed similarity in GA genotype with Chinese, Japanese and Korean populations and is higher than Caucasian, American and Indian populations. The frequency of 2677G allele is the lowest in India and is the highest in Brazil, Slovenia, Polish and our population.

In a study, McDonald and colleagues discussed the role of PGP in limiting

brain absorption of ivermectin pesticides. They expressed 2677T is the prevalent non-synonymous human SNP, while 2677A occurs at a much lower allelic frequency (2).

As it is seen in Table 4, the frequency of 2677A allele is the lowest in Czech and it is seen mostly in Asian countries. The frequency of 2677T allele is the lowest in our population and is the highest in India. Potocnik and colleagues have evidenced that a higher frequency of T allele in position 2677 of *MDR1* was indicated in patients with colorectal cancer when compared with healthy subjects (38). They showed that G2677T/A polymorphism correlated with altered expression of PGP and activity in colon tissue. In another study,

Table 4 Comparison of genotype frequencies of MDR1 G2677T/A polymorphism reported for Iranian population with other populations published.

Population	n	Genotype frequency						Reference	
		GG	GT	TT	GA	AA	TA		
Asian	Iranian (Mazandaran province)	120	0.65	0.208	0	0.141	0	0	This study
	Indian	87	0.138	0.310	0.414	0.081	N.d	0.58	[26]
	Chinese	200	0.175	0.375	0.210	0.110	0.025	0.105	[25]
	Japanese	154	0.195	0.318	0.182	0.149	0.026	0.130	[24]
	Korean	632	0.191	0.339	0.163	0.155	0.035	0.117	[23]
Caucasion	British	285	0.329	0.474	0.147	0.025	N.d	0.025	[32]
	Polish	204	0.387	0.397	0.176	0.020	N.d	0.020	[31]
	Serbian	158	0.26	0.52	0.15	0.03	0	0	[15]
	Czech	189	0.296	0.471	0.222	0.05	N.d	N.d	[30]
	Bulgarian	160	0.344	0.431	0.225	N.a	N.a	N.a	[29]
	Scottish	370	0.276	0.470	0.254	N.a	N.a	N.a	[28]
	Slovenian	355	0.375	0.445	0.18	N.a	N.a	N.a	[27]
	America	Brazil	106	0.400	0.400	0.180	0.020	N.d	N.d

G: wild-type allele, T, A: mutant allele, n: number of subjects.

ND : not detected. NA : not analyzed.

Sapmaz and colleagues investigated the G2677T/A polymorphism in the *MDR1* gene in Turkish patients with inflammatory bowel disease and a healthy control group. They concluded that G2677T/A polymorphism was not found to be a risk factor for Crohn's disease or ulcerative colitis (39).

Penna and colleagues examined *MDR1* polymorphism G2677T in B-chronic lymphocytic leukemia in Italian Population. They observed higher T allele frequency in patients with B-CLL when compared with controls (16). Heterozygous genotype may lead to a different of the encoded protein and mRNA expression. Zhou and colleagues investigated the association between

G2677T/A polymorphism and chemosensitivity of paclitaxel in Chinese advanced gastric cancer patients. They concluded that G2677T/A polymorphisms can affect the chemosensitivity of paclitaxel and responsiveness (40). Another study conducted by Ichihara and colleagues examined the association of this polymorphism of *MDR1* with obesity in Japanese individuals (41). They claim that the G2677T/A polymorphism of *MDR1* was significantly associated with the prevalence of obesity. G2677T/A polymorphism may be related with deep changes in the levels of hormones or other physiological molecules.

In conclusion, our study established the frequency of *MDR1* G2677T/A polymorphism in the population from Mazandaran province of Iran. Considering the number and significance of PGP substrates, determination of the frequency of functionally important SNPs in the *MDR1* gene provides useful data for the evaluation of inter-individual differences in drug response. Moreover, these data can be used for the prediction of any adverse effects and the possibility of adverse reactions during treatment with PGP-substrate drugs in patients residing in our province. The recent identification of multiple SNPs in the *MDR1* gene and in other genes (such as *CYP3A4/5*, *CYP2D6*, *CYP2C19*, *NAT2* and *UGT1A1*) that are involved in drug

metabolism provides an opportunity for the development of molecular tools for drug and dose modification for each patient. However, the relative importance of variability in PGP function due to exogenous and genetic factors for drug disposition, therapeutic outcome and disease risk needs to be clarified in future studies in our province.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Deen M, Vries E, Timens W, Scheper RJ, Timmer-Bosscha H, Postma DS. ATP-binding cassette (ABC) transporters in normal and pathological lung. *Respir Res* 2005;6:59.
2. Macdonald NE, Gledhill AL. Potential impact of ABCB1 (p-glycoprotein) polymorphisms on avermectin toxicity in humans. *Arch Toxicol* 2007;81:553-63.
3. Horinouchi M, Sakaeda T, Nakamura T, Morita Y, Tamura T, Aoyama N, et al. Significant genetic linkage of MDR1 Polymorphisms at positions 3435 and 2677: Functional relevance to pharmacokinetics of digoxin. *Pharmaceutical* 2002;19:1581-5.
4. Ambudkar S.V, Dey S, Hrycyna C.A, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu. Rev Pharmacol Toxicol* 1999;39:361-98.
5. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol Ther* 2006;112:457-73.
6. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham M.C. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 1987;84:7735-8.
7. Hartmann G, Kim H, Piquette-Miller M. Regulation of the hepatic multidrug resistance gene expression by endotoxin and inflammatory cytokines in mice. *Int Immunopharmacol* 2001;1:189-99.
8. Arceci RJ. Clinical significance of P-glycoprotein in multidrug resistance malignancies. *Blood* 1993;81:2215-22.
9. Fromm FM. Genetically determined differences in P-glycoprotein function: implications for disease risk. *Toxicology* 2002;181-2:299-300.
10. Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, Von Richter O, Zundler J, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 1999;104:147-53.
11. Fromm M.F, Kim R.B, Stein C.M, Wilkinson G.R, Roden DM. Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 1999;99:552-7.
12. Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, et al. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. *Clin Pharmacol Ther* 2000;68:345-55.
13. Fromm F.M. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans.

- Advanced Drug Delivery Reviews 2002;54:1295-310.
14. Sakaeda T, Nakamura T, Okumura K. MDR1 genotype-related pharmacokinetics and pharmacodynamics. *Biol Pharm Bull* 2002;25:1391-400.
 15. Milojkovic M, Stojnev S, Jovanovic I, Ljubisavljevic S, Stefanovic V, Sunder-Plassman R. Frequency of the C1236T, G2677T/A and C3435T MDR1 gene polymorphisms in the Serbian population. *Pharmacol Rep* 2011;63:808-14.
 16. Penna G, Allegra A, Alonci A, Aguenouz M, Garufi A, Cannavo A, et al. MDR-1 polymorphisms (G2677T and C3435T) in B-chronic lymphocytic leukemia: an impact on susceptibility and prognosis. *Med Oncol* 2010;28:1549-54.
 17. Al-Mohizea AM, Alkharfy K.M, Bagulb K.M, Alghamdi AM, Al-Jenoobi FI, Al-Muhsen S, et al. Genetic variability and haplotype profile of MDR1 in Saudi Arabian males. *Mol Biol Rep* 2012;39:10293-301.
 18. Mickley LA, Lee JS, Weng Z, Zhan Z, Alvarez M, Wilson W, et al. Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells unselected and drug-selected cell lines, and human tumors. *Blood* 1998;91:1749-56.
 19. Kim R.B, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001;70:189-99.
 20. Wasilewski A, Zalewski G, Chyczewski L, Zoch-Zwierz W. MDR-1 gene polymorphisms and clinical course of steroid-responsive nephrotic syndrome in children. *Pediatr Nephrol* 2007;22:44-51.
 21. Chen B, Fang J, Zhang W, Jin Z, Yu Z, Cai W. Detection of C1236T, G2677T/A, and C3435T polymorphism of MDR1 by amplification refractory mutation system PCR. *J Clin Lab Anal* 2009;23:110-16.
 22. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in Human Placenta: Relation to Genetic Polymorphism of the Multidrug Resistance (MDR)-1 Gene. *J Pharmacol Exp Ther* 2001;297:1137-43.
 23. Lee SS, Kim SY, Kim WY, Thi-Le H, Yoon YR, Yea SS, et al. MDR1 genetic polymorphisms and comparison of MDR1 haplotype profiles in Korean and Vietnamese populations. *Ther. Drug Monit* 2005;27:531-5.
 24. Komoto C, Nakamura T, Sakaeda T, Kroetz D.L, Yamada T, Omatsu H, et al. MDR1 haplotype frequencies in Japanese and Caucasian and Japanese patients with colorectal cancer and esophageal cancer. *Drug Matab. Pharmacokinet* 2006;21:126-32.
 25. Zhang Y, Jiang XH, Hu YQ, Li ZR, Su L, Wang ZG, Ma G. MDR1 genotypes do not influence the absorption of a single oral dose of 600 mg valacyclovir in healthy Chinese Han ethnic males. *Br J Clin Pharmacol* 2008;66:247-54.
 26. 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 haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics* 2003;13:89-95.
 27. Potocnik U, Glavac D, Dean M. Common germline MDR1/ABCB1 functional polymorphisms and haplotypes modify susceptibility to colorectal cancers with high microsatellite instability. *Cancer Genet Cytogenet* 2008;183:28-34.
 28. Ho GT, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, et al. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology* 2005;128:288-96.
 29. Petrova DT, Nedeva P, Maslyukov S, Toshev S, Yaramov N, Atanasova S, et al. No association between MDR1 (ABCB1) 2677G>T and 3435C>T polymorphism and sporadic colorectal cancer among Bulgarian patients. *J. Cancer Res. Clin Oncol* 2008;134:317-22.
 30. Pechandova K, Buzkova H, Slanar O, Perlik F. Polymorphisms of the MDR1 gene in the Czech population. *Folia Biol* 2006;52:184-9.
 31. Kurzawski M, Pawlik A, Gornik W, Drozdziak M. Frequency of common MDR1 gene variants in a Polish population. *Pharmacol Rep* 2006;58:35-40.
 32. Onnie CM, Fisher SA, Pattni R, Sanderson J, Forbes A, Lewis CM, et al. Associations of the multidrug resistance gene (ABCB1 or MDR1) and inflammatory bowel disease and their effects on disease behavior: a case-control and meta-analysis study. *Inflamm Bowel Dis* 2006;12:263-71.
 33. Estrela RC, Ribeiro FS, Carvalho RS, Gregorio SP, Diasneto E, Struchiner CJ, et al. Distribution of ABCB1 polymorphisms among Brazilians: impact of population admixture. *Pharmacogenomics* 2008;9:267-76.
 34. Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response. *Ann Rev Genom Hum Genet* 2001;2:9.
 35. Rusnak JM, Kisabeth RM, Herbert DP, McNeil DM. Pharmacogenomics: a clinician's primer on emerging technologies for improved patient care. *Mayo Clin Proc* 2001;76:299-309.
 36. Roses AD. Pharmacogenetics and the practice of medicine. *Nature* 2000;405:857-65.
 37. Vesell ES. Advances in pharmacogenetics and pharmacogenomics. *J Clin Pharmacol* 2000;40:930.
 38. Potocnik U, Ravnik-Glavac M, Glavac D. Functional MDR1 polymorphisms (G2677T and C3435T) and TCF4 mutations in colorectal tumors with high

- microsatellite instability. *Cell Mol Biol Lett* 2002;7:92-5.
39. Sapmaz A, Ozen Karatayli SC, Dagli U, Kilic ZM, Toruner M, Celik Y, et al. Effects of polymorphism in G2677T/A triallelic region of MDR1 gene in Turkish patients with inflammatory bowel disease. *Turk J Gastroenterol* 2008;19:168-73.
40. Zhou J, Deng W, Gao J, Yuan J, Li Y, Shen L. Association between ABCB1 G2677T/A polymorphisms and chemosensitivity of paclitaxel in advanced gastric cancer. *Zhonghua Wei Chang Wai Ke Za Zhi (Chinese Journal of Gastrointestinal Surgery)* 2015;18:123-6.
41. Ichihara S, Yamada Y, Kato K, Hibino T, Yokoi K, Matsuo H, et al. Association of a polymorphism of ABCB1 with obesity in Japanese individuals. *Genomics* 2008;91:512-16.