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Frequency of *MDR1* single nucleotide polymorphisms in a Jordanian population, including a novel variant

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ABSTRACT. The multidrug resistance gene (*MDR1* or *ABCB1*) codes for P-glycoprotein, which plays an important role in regulating absorption, distribution, and elimination of drugs. We examined *MDR1* gene variants in 100 unrelated subjects from various regions of Jordan. The *MDR1* gene was scanned using direct sequencing. Six rare variants in *MDR1* were detected, including a new variant, T3075A. This variant did not affect the protein sequence (synonym for threonine). Among the common SNPs, the frequencies of rs1128503 (C1236T) genotypes were: 0.23 (CC), 0.41 (CT) and 0.36 (TT). For the rs2032582 (G2677T) SNP, genotype frequencies were 0.38 for GG, 0.45 for GT, 0.13 for TT, 0.03 for GA, and 0.01 for TA, whereas for rs1045642 (C3435T), genotype frequencies were 0.17 for CC, 0.5 for CT and 0.33 for TT. The observed distribution of the common variants in the Jordanian population was within the range detected in other populations. These data on *MDR1* gene variants in the Jordanian population will be useful for investigations on response to P-glycoprotein substrate drugs.

Key words: *MDR1*; SNP; Jordan; Allele; Gene

INTRODUCTION

Pharmacogenetics - the study of genetic variation and its effects on drug response - is expected to play a key role in individualizing patient care through "personalized medicine" (Roses, 2008). Pharmacogenetic investigations usually focus on drug-metabolism genes involved in specific disease. The multi-drug resistance gene (*MDR1* or *ABCB1*) spans 28 exons on chromosome 7; its cDNA consists of 3843 bp (Fojo et al., 1986). *MDR1* encodes P-glycoprotein (P-gp), which is a large, 170-kD transmembrane protein composed of 2 homologous halves, each of which contains 6 transmembrane domains and an intercellular binding site for ATP; thus, it functions as an ATP-dependent efflux pump (Higgins et al., 1997). It was first detected in cancer cells where it is responsible for multiple resistance to anticancer agents (He et al., 2011); however, it is also highly expressed in normal tissues such as the brain, liver, kidney, lymphocytes, placenta, gut, and testes (Cascorbi, 2011). P-gp plays an important role in regulating drug absorption, distribution, and elimination. It mediates the energy-dependent efflux of xenobiotics in epithelial tissues throughout the human body including the intestinal mucosa, liver canalicular membrane, and kidney proximal tubules, as well as blood-tissue barriers such as the brain and placenta (He et al., 2011). P-gp efflux, therefore, may decrease intestinal absorption, increase biliary excretion and renal tubular secretion, and impair distribution of various drugs to the brain.

P-gp exhibits wide substrate specificity for structurally different drugs and thus mediates resistance to a variety of drugs including anti-arrhythmics, antifungals, calcium channel blockers, chemotherapeutic agents, hormones, immunosuppressants, and HIV-protease inhibitors (Kuypers et al., 2008). Because P-gp is found in tissues important for drug disposition, variations in expression and function of P-gp due to MDR1 polymorphisms may influence drug pharmacokinetics and therapeutic efficacy.

MDR1 gene expression is highly variable between subjects from the same as well as different races, and many variants have been identified (Hattori et al., 2007; Sipeky et al., 2011). For example, among Caucasians, the C3435T polymorphism in exon 26 correlates with expression of P-gp in the intestine, and people who are homozygous for the T allele show more than 2-fold lower duodenal P-gp protein expression levels compared with C homozygotes. Since concentrations of P-gp in the intestine determine the extent of drug absorption, genotype-related differences in bioavailability are seen for several drugs (e.g., digoxin) (Chen et al., 2011; Li et al., 2011; Yan et al., 2011; Ponnala et al., 2012). Substantial differences in allele frequencies have been reported in different racial groups. For example, significantly higher frequencies of the C/C genotype of C3435T SNP were reported in West Africans and African Americans than in White Americans (Chelule et al., 2003; Lewis et al., 2007). In this study, we scanned the *MDR1* gene to identify variants in the Jordanian population. In addition, the distribution of common variants was compared to that reported in other populations.

MATERIAL AND METHODS

Subjects

Unrelated subjects of both sexes (N = 100) were recruited randomly from different

regions of Jordan. Random selection was carried out based on geographical distribution and population density. All participants were asked to provide written informed consent. The study was approved by the Institutional Review Board (IRB) of the Jordan University of Science and Technology.

DNA extraction

Blood samples (5 mL) were collected in EDTA tubes for DNA extraction. Genomic DNA was extracted with the Wizard DNA Extraction Kit (Promega, Madison, USA) according to the manufacturer protocol. DNA concentrations were measured using a SmartSpect™ 3000 (Bio-Rad, Hertfordshire, UK) and samples were stored at -20°C prior to use.

PCR amplification of *MDR1* axons

Amplification of *MDR1* exons was carried out as previously described (Kim et al., 2001) with the following modifications: for exon 8, the following primers were used: F: 5'-TAGCGTATGCAAAAGCTGGA-3' and R: 5'-TCTGAAGGGCATTTGAGAAGA-3', and for exon 18, F: 5'-CCAGGATGGGTTCTTCACTG-3' and R: 5'-CCCCCAGTTGAATAATGA TG-3'. In each reaction, approximately 100 ng genomic DNA, 1 µM of each forward and reverse primer, and green PCR master mix (Promega) were used in a final reaction volume of 25 µL. PCR products were detected after electrophoresis on 2% agarose.

Sequencing of MDR1 allelic variants

Purification of PCR products was carried out using the PCRquick-Spin™ PCR Product Purification system as described by the manufacturer (INTro Biotechnolgy, Korea).

The purified PCR products were fully sequenced using the Big Dye Terminator Cycle Sequencing Kit version 3.1 (QIAquick, Germany). The ABI 3700 DNA Analyzer (Applied Biosystems) was used for sequencing. DNA sequences were compared to the reference sequence (NM_000927.4) stored in the Ensembl Genome Browser (http://www.ensembl.org/index.html). Presence of a gene variant was confirmed by sequencing of the reverse strand. Sequencing results were analyzed using the ChromasPro version 1.34 software (http://www.technelysium.com.au/ChromasPro.html). Finally, the effect of the identified novel variant was analyzed with the STARORF software (Massachusetts Institute of Technology, UK).

Statistical analysis

Genotype distributions were analyzed for Hardy-Weinberg equilibrium. The SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. P values smaller than 0.05 were considered to be significant.

RESULTS

Jordan is a small country in Southwest Asia and is classified as a low-income country.

The population is predominantly Arab (98%), mostly urban (70%) (Khabour et al., 2009). As of the 2010 census, the total population of Jordan was 6.13 million.

In this study, we performed a complete analysis of *MDR1* coding exon sequences (2-28) in 100 DNA samples obtained from health subjects recruited from different parts of Jordan. Table 1 shows the rare detected variants and their frequencies. Four of the rare variants are synonymous mutations and the remaining are missense mutations. All rare variants were detected in heterozygous genotypes.

Table 1. Frequencies of *MDR1* gene variants in the Jordanian population.

SNP identification No.	mRNA position	Variation	Function	Allele frequency
Rs28364274	4244	A>G	Missense: Ile>Val	0.005
rs41309228	3903	G>T	Missense: Ser>Ile	0.005
This study	3075	T>A	Synonymous: Thr	0.005
rs138926696	2332	T>C	Synonymous: Asp	0.005
rs2229109	1692	G>A	Missense: Ile>Asn	0.005
rs1128502	1048	A>T	Synonymous: Gly	0.005
rs115493381	652	G>T	Synonymous: Val	0.005

Table 2 shows the allele and genotype frequencies of common *MDR1* variants in the study sample. The genotype frequencies for rs1128503 (C1236T) were 0.23 (CC), 0.41 (CT), and 0.36 (TT); for rs2032582 (G2677T) they were 0.38 (GG), 0.45 (GT), 0.13 (TT), 0.03 (GA), and 0.01 (TA); for rs1045642 (C3435T) they were 0.17 (CC), 0.5 (CT), and 0.33 (TT). All common *MDR1* genotypic groups were in Hardy-Weinberg equilibrium ($P > 0.05$).

Table 2. Genotype and allele frequencies of *MDR1* gene SNPs in the Jordanian population.

Polymorphism	Genotypes (%)	Alleles (%)	Hardy-Weinberg P
rs1128503 (C1236T)	CC (23)	C (43.5)	
	CT (41)	T (56.5)	
	TT (36)		0.10
rs2032582 (G2677T)	GG (38)	G (62)	
	GT (45)	T (36)	
	GA (3)	A (2)	
	TT (13)		
	TA (1)		
	AA (0)		0.98
rs1045642 (C3435T)	CC (17)	C (42)	
	CT (50)	T (58)	
	TT (33)		0.79

Only one novel variant was detected in a heterozygous sample. This variant (U3075A) is a silent mutation located in exon 21 (synonymous: Thr; Figure 1).

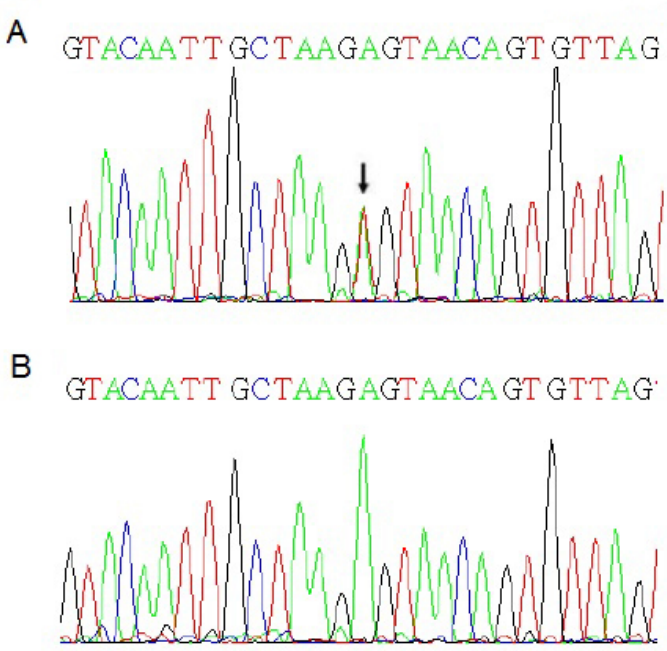

Figure 1. Nucleotide sequence analysis showing T3075A variant in exon 21 of the *MDR1* gene. The identified novel variant (T3075A) is a synonymous mutation located in exon 21. The arrow indicates the position of the variant. **A.** Heterozygous TA genotype. **B.** Homozygous TT genotype.

DISCUSSION

The ability to predict a patient's drug response based on their genetic information is emerging as a solution to reduce adverse events and/or improve therapeutic efficacy. Targeting therapies to patients who are most likely to benefit with minimal adverse events will improve patient care and facilitate the approval of new, innovative medicines (Roses, 2008; Katz and Bhathena, 2009). Furthermore, advances in pharmacogenetic working models is associated with superior individualized care for patients suffering from every condition, especially those with cancer (van Schaik, 2008), cardiovascular diseases (D'Andrea et al., 2008), asthma (Hawkins and Peters, 2008), diabetes (Vella and Camilleri, 2008), and psychiatric or neurological disorders (Kadiev et al., 2008). In this study, we scanned the *MDR1* coding sequences to identify variations. Three common and 6 rare previously known SNPs were detected. In addition, a novel variant in exon 21 was reported for the first time.

The 3 common SNPs in the Jordanian population were rs1128503, rs2032582, and rs1045642. These polymorphisms are also common in other human populations but with interethnic differences (Table 3), indicating the ancient origin of these polymorphisms in human history. The C and T alleles of rs1128503 are abundant in the Jordanian population with frequencies of 43.5 and 56.5%. The distribution of this SNP is similar to that reported in the Turkish population (Gumus-Akay et al., 2008). The T allele is significantly less frequent in Serbian, German, and Russian populations (Gaikovitch et al., 2003; Cascorbi, 2011; Milojkovic et al.,

2011) and more frequent in Japanese and Chinese populations (Komoto et al., 2006; Zhang et al., 2008) (see Table 3 for a summary). Similar distributions were also found for the C and T alleles of rs1045642 with frequencies of 42 and 58%. This distribution is similar to that reported in European populations (Gaikovitch et al., 2003; Turgut et al., 2006; Cascorbi, 2011; Milojkovic et al., 2011) but different from that observed in Japanese and Chinese (Komoto et al., 2006; Zhang et al., 2008). The G allele of rs2032582 is enriched in the Jordanian population with 62 *vs* 36% for the T allele and 2% for the A allele. As with rs1045642, this distribution is similar to that reported in European populations (Gaikovitch et al., 2003; Turgut et al., 2006; Cascorbi, 2011; Milojkovic et al., 2011) but different from that observed in Japanese and Chinese populations (Komoto et al., 2006; Zhang et al., 2008). This could be due to enrichment of the allele A in the Japanese and Chinese ethnicities. Thus, according to the distribution of these common SNPs, the Jordanian population is more closely related to Caucasian than Asian populations.

Table 3. Summary of the distribution of rs1128503 SNP of the *MDR1* gene among various populations.

Variable	C3435T		G2677T			T1236C	
	Allele C	Allele T	Allele G	Allele T	Allele A	Allele C	Allele T
Jordan (the current study)	42	58	62	36	2	43.5	56.5
Serbian (Milojkovic et al., 2011)	47	53	53	43	4	54	46
German (Cascorbi et al., 2001)	46	54	56.5	41.5	3	59.5	40.5
Russian (Gaikovitch et al., 2003)	45.5	54.5	54.5	42	3.5	52	48
Turkish (Turgut et al., 2006; Gumus-Akay et al., 2008)	46.5	53.5				47.5	52.5
Japanese (Komoto et al., 2006)	59.5	40.5	42.5	40.5	17	34.5	65.5
Chinese (Zhang et al., 2008)	56.5	43.5	42	44.5	14.5	34.5	65.5

The most well studied variant of *MDR1* is rs1045642 (C3435T). Although this SNP does not affect the protein sequence of P-glycoprotein, the variant affects expression of the *MDR1* gene in the intestine. In addition, studies have indicated that rs1045642 is associated with variable response to chemical therapy. For example, a meta-analysis suggested a correlation between rs1045642 and tacrolimus pharmacokinetics (Li et al., 2012). Other studies connect this SNP with therapeutic outcomes in gastric, lung, blood, and other cancers (Li et al., 2011; Yan et al., 2011) and responses to anti-epileptic (Ponnala et al., 2012) and rheumatoid arthritis drugs (Chen et al., 2011). Moreover, rs1045642 is associated with the risk of breast, renal, blood, and other cancers (Mhaidat et al., 2011; Qian et al., 2012; Wang et al., 2012). Similarly, rs1128503 is very common and influences imatinib response in patients with chronic myeloid leukemia, tacrolimus response in liver transplant patients (Ni et al., 2011; Yu et al., 2011), and risk of ulcerative colitis (Huebner et al., 2009). Finally, the clinical significance of rs1045642 is evidenced by its association with response to drugs such as cyclosporine and docetaxel (Pan et al., 2009; Wang et al., 2009; Kasuya et al., 2012) and increased colorectal cancer risk (Potocnik et al., 2008). Thus, determination of the frequency of these clinically important SNPs in the *MDR1* gene is important for prescribing and decreasing the likelihood of adverse reactions and side effects during treatment. In this study, we reported a new *MDR1* variant, a silent T3075A substitution. The effect of this variant on the function of MDR1 was not investigated; however, since the mutation is silent, we do not expect a significant effect on the MDR1 protein function.

Jordan is located in the corner of 3 continents. This geographical location put Jordan on the major trade route between east and west. In addition, the area was exposed to several occupational waves from west and east. This made the Jordanian population open to mixing with other European, African, and Asian populations. Therefore, we expected to find a distribution of *MDR1* SNPs in the Jordanian population between those observed in other populations. The results of this study support this hypothesis.

This study demonstrated the distribution of *MDR1* variants in Jordanians and identified a novel polymorphism. This could form the foundation of future investigations of the relationships between *MDR1* polymorphisms and various diseases and drug responses.

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