DNA AND CELL BIOLOGY
Volume 32, Number 10, 2013

Pp. 582–588
DOI: 10.1089/dna.2013.2108

Genotype Variability and Haplotype Frequency of *MDR1* (*ABCB1*) Gene Polymorphism in Morocco

Yaya Kassogue,[1] Hind Dehbi,[1] Sanaa Nassereddine,[1] Meryem Quachouh,[2] and Sellama Nadifi[1]

The multidrug resistance gene (*MDR1*) plays an important role in the transport of a wide range of drugs and elimination of xenobiotics from the body. Identification of polymorphisms and haplotypes in the *MDR1* gene might not only help understand pharmacokinetics and pharmacodynamics of drugs, but also can help in the prediction of drug responses, toxicity, and side effects, especially, in the era of personalized medicine. We have analyzed the genotypic and haplotypic frequencies of the three most common single-nucleotide polymorphisms in the *MDR1* gene in a sample of 100 unrelated healthy Moroccan subjects by polymerase chain reaction–restrictive fragment length polymorphism. The observed genotype frequencies were 43% for 1236CC, 49% for 1236CT, and 8% for 1236TT in exon 12; 49% for 2677GG, 47% for 2677GT, and 4% for 2677TT in exon 21; 39% for 3435CC, 51% 3435CT for 3435TT, and 10% for 3435TT in exon 26, respectively. We found that all polymorphisms were in Hardy–Weinberg equilibrium. Moderate linkage disequilibrium (LD) was observed between the three polymorphisms, the strongest LD in our study has been observed between C1236T and G2677T ($D' = 0.76$; $r^2 = 0.45$). We identified eight haplotypes, the most frequent were 1236C-2677G-3435C (53%), 1236T-2677T-3435T (21%), and 1236C-2677G-3435T (10%), respectively. Our findings might facilitate future studies on pharmacokinetics of P-glycoprotein substrate drugs and interindividual variability to drugs in Moroccan patients.

Introduction

The human multidrug resistance gene (*MDR1*), expressed in most of the vital organs, plays an important role in the body protection against xenobiotics. This gene with 28 exons located on chromosome 7q21.12 is composed of 1280 amino acids, and it encodes a transmembrane protein of 170-kDa named P-glycoprotein (P-gp) (Ueda *et al.*, 1987). P-gp is a member of the adenosine triphosphate banding cassette (ABCB1) superfamily, which constitutes an integral part of the blood–brain barrier and functions as a drug-transport pump carrying a variety of drugs from the brain back into the blood. The development of simultaneous resistance to a number structurally independent drug is a major constraint to the chemotherapy of cancer (Shen *et al.*, 1986). It is noticed that both tumor and normal tissues express the P-gp, but a higher level is observed in the adrenal gland and the kidney; an intermediate level in the lung and liver; and a lower level in the jejunum, colon, and rectum (Fojo *et al.*, 1987). P-gp transports actively amphipathic molecules across lipid membranes; it can influence bioavailability and therapeutic outcome of drugs (Fromm, 2002). Drugs used in the treatment of cancer, HIV, epileptic and cardiac diseases as well as environmental carcinogens are some substrates transported by P-gp (Marzolini *et al.*, 2004). Nowadays, more than fifty single-nucleotide polymorphisms (SNPs) and 3 insertions/deletions located at exonic or intronic positions, synonymous or nonsynonymous, have been described in the *MDR1* gene (Ishikawa *et al.*, 2004). The functional involvement of most of these SNPs remains unknown, although certain SNPs have been characterized on a functional level and have been found to be associated with impaired transport function and expression. The C1236T (exon 12; rs1128503, Gly412Gly), G2677T/A (exon 21; rs2032582, Ala893Ser/Thr), and C3435T (exon 26; rs1045642, Ile1145Ile) are the most commonly studied. It was found that the homozygous mutant 3435TT in exon 26 was associated with higher plasma levels of digoxin compared to the wild-type 3435CC (Hoffmeyer *et al.*, 2000). Similar findings were obtained with fexofenadine in 3435TT/2677TT haplotype carriers (Shon *et al.*, 2005). Furthermore, other authors failed to find any association between plasma levels and these two SNPs with the same drugs (Kim *et al.*, 2001; Cavaco *et al.*, 2003). The possible modification of mRNA stability by 3435C>T SNP (Wang *et al.*, 2005) or linkage disequilibrium (LD) between this SNP and other SNPs, particularly 2677G>T in exon 21 was advanced to explain this discrepancy in P-gp expression (Cavaco *et al.*, 2003; Gümüş-Akay *et al.*, 2010; Al-Mohizea *et al.*, 2012). Indeed, recent investigations have highlighted not only the importance of haplotype analysis of the *MDR1* gene in drug pharmacokinetics, but also their involvement in the development of certain diseases and in the response to drugs in several ethnic groups (Hoffmeyer *et al.*, 2000; Kim *et al.*, 2006;

[1]Genetics and Molecular Pathology Laboratory, Medical School of Casablanca, University Hassan II, Casablanca, Morocco.
[2]Department of Onco-Hematology, Ibn Rochd University Hospital, Casablanca, Morocco.

Dulucq *et al.*, 2008; Ekhart *et al.*, 2009; Vivona *et al.*, 2012). To the best of our knowledge, there are no data about the polymorphisms of the *MDR1* gene in our population. So, we sought to assess the frequency of C1236T, G2677T/A, and C3435T polymorphisms and determine the haplotypes with the aim of providing useful findings, which might help future studies on bioavailability, pharmacokinetics of various drugs, and interindividual variability to drugs in Moroccan patients.

Materials and Methods

Subjects

Four milliliters of peripheral venous blood have been collected in the EDTA tube at the laboratory of genetic and molecular diseases, Faculty of Medicine, the Hassan II University in Casablanca on a total of 100 unrelated healthy participants (68 females and 32 males). The subjects were composed of Arabs (71%, $n=71$) and Berbers (29%, $n=29$) with a mean age of 31.78 ± 12.94 years (range 18–77 years). The study was approved by the local Ethics Committee, and each participant agreed and signed informed consent.

Genotyping of MDR1 *polymorphisms*

Genomic DNA was extracted from whole blood by the salting-out method previously described by Miller and Polesky (1988). The genotyping of the three SNPs was done by polymerase chain reaction–restrictive fragment length polymorphism (PCR-RFLP). The mixture consisted of 100 ng of DNA, 1×of 5×GoTaq Flexi Buffer (Promega), 1.25 mM MgCl2, 0.2 mM of each dNTP, 0.625 μM of each primer, 0.5 U Go Taq DNA polymerase (Promega) completed with sterile water to 25 μL. The primers used for C1236T (exon 12), G2677T/A (exon 21), and C3435T (exon 26) summarized in Table 1 were previously published by Cascorbi *et al.* (2001) and Tanabe *et al.* (2001). The PCR amplification was performed with an initial denaturation at 94°C for 5 min followed by 35 cycles of three steps: denaturation at 95°C for 90 s, annealing at 55°C for 60 s, extension at 72°C for 90 s, and a final extension at 72°C for 7 min. Negative controls (tube without DNA) were included in all reactions. PCR products were run on a 1% agarose gel (expected sizes are summarized in Table 1); the digestion was carried out with 10 units of restriction enzymes *Hae*III, *Ban*I, *Bsr*I, and *Mbo*I for 16 h. The digestion products were analyzed on a 3% agarose gel stained with 0.5 μg/mL ethidium bromide (Figs. 2, 3, and 4). The enzymatic restriction conditions and digestion product sizes are summarized in Table 1.

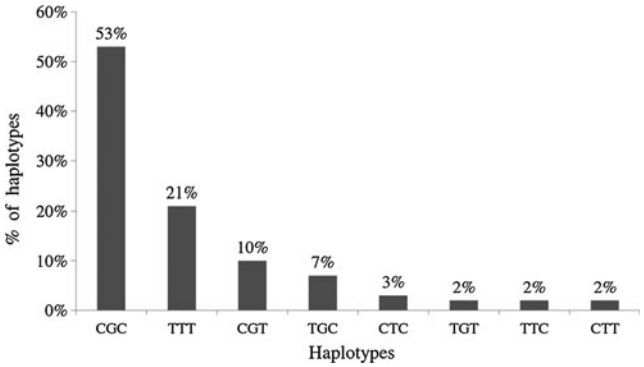


FIG. 1. Haplotype distribution in the Moroccan population from exon 12 (C1236T), exon 21 (G2677T/A), and exon 26 (C3435T) of the *MDR1* gene.

Statistical analysis

The statistical package SPSS version 16 (SPSS, Inc.) has been used to estimate possible combinations between different genotypes. SNPAnalyzer 2.0 was used to determine the allelic and genotypic frequencies as well as for the Hardy–Weinberg equilibrium test. A p value less than 0.05 was considered as significant. Expectation maximum algorithm from the same SNPAnalyzer 2.0 was used to identify haplotypes and establish their frequencies and finally to do LD analysis between SNPs (Yoo *et al.*, 2008).

Table 1. The Primer Sequences and PCR-RFLP Conditions for the Identification of the Three SNPs in the *MDR1* gene

Exon	*SNP*	*Primers sequences 5′ 3′*	*Amplicon size (bp)*	*Enzymes*	*T°*	*RFLP product size (bp)*	*Reference*
12	C1236T	TATCCTGTGTCTGTGAATTGCC CCTGACTCACCACACCAATG	269	*Hae*III	37°C	[a]CC: 269 + 62 + 35 [b]CT: 269 + 97 + 62 + 35 [c]TT: 269 + 97	Cascorbi *et al.* (2001)
26	C3435T	TTGATGGCAAAGAAATAAAGC CTTACATTAGGCAGTGACTCG	206	*Mbo*I	37°C	[a]CC: 130 + 76 [b]CT: 206 + 130 + 76 [c]TT: 206	Tanabe *et al.* (2001)
21	G2677T	TGCAGGCTATAGGTTCCAGG TTTAGTTTGACTCACCTTCCCG	224	*Ban*I	37°C	[a]GG: 198 + 26 [b]GT: 224 + 198 + 26 [c]TT: 224	Cascorbi *et al.* (2001)
21	G2677A	TGCAGGCTATAGGTTCCA GG GTTTGACTCACCTTCCCAG	220	*Bsr*I	65°C	[a]GG206 + 14 [b]GA: 220 + 206 + 14 [c]AA: 220	Cascorbi *et al.* (2001)

[a]Wild-type homozygote.
[b]Wild/variant heterozygote.
[c]Homozygote mutant variant.
T°, digestion temperature; PCR-RFLP, polymerase chain reaction–restrictive fragment length polymorphism; SNP, single-nucleotide polymorphisms.

Table 2. The Allelic Frequency and Genotype Percentage of *MDR1* C1236T, G2677T/A, and C3435T in 100 Healthy Subjects

SNP	*Exon*	*Allele (%)*			*Genotype (%)*					χ^2	p-*Value*
C3435T	26	C	T		CC	CT	TT			1.3	0.25
		64.5	35.5		39	51	10				
G2677T/A	21	G	T	A	GG	GT	TT	GA	AA	3.19	0.07
		72.5	27.5	0	49	47	4	0	0		
C1236T	12	C	T		CC	CT	TT			1.36	0.24
		67.5	32.5		43	49	8				

χ^2, Chi-square (Hardy–Weinberg test with df = 1); df, degree of freedom.

Results

In this study, 100 healthy individuals have participated, allelic and genotypic frequencies of C1236T, G2677T, and C3435T of the *MDR1* gene are summarized in Table 2. The observed genotype frequencies were 43%, 49%, and 8% for 1236CC, 1236CT, and 1236TT in exon 12; 49%, 47%, and 4% for 2677GG, 2677GT, and 2677TT in exon 21, respectively. However, no variant of G2677A was observed. In exon 26, genotypic frequencies were 39% for 3435CC, 51% for 3435CT, and 10% for 3435TT, respectively. The observed allelic and genotypic frequencies did not deviate from those expected at Hardy–Weinberg equilibrium (Table 2). The relative frequencies of different combinations between the three SNPs are summarized in Table 3, we found out that 27%–35% of our subjects were wild type, 32%–35% were heterozygous, and 3%–4% were mutants when SNPs were considered in pairwise combinations. The LD between the three SNPs was moderate, the LD (D′) and Pearson correlation coefficient (r^2) between C1236T and G2677T were 0.76 and 0.45; between C3435T and G2677T were 0.72 and 0.36; between C1236T and C3435T were 0.55 and 0.26 (Table 3). Of the 100 subjects, 8 haplotypes were observed; the three most common haplotypes were 1236C-2677G-3435C (53%), 1236T-2677T-3435T (21%), and 1236T-2677G-3435T (10%) (Fig. 1).

Table 3. Frequency of Different Genotype Combinations Between C1236T, C3435T, and C2677T Polymorphisms By Blocks

	2677GG		*2677GT*		*2677TT*			
Block 1	N	f	N	f	N	f	D′	r^2
3435CC	**32**	**0.32**	7	0.07	0	0		
3435CT	16	0.16	**34**	**0.34**	1	0.01	0.72	0.36
3435TT	1	0.01	6	0.06	**3**	**0.03**		
	1236CC		*1236CT*		*1236TT*			
Block 2	N	f	N	f	N	f	D′	r^2
3435CC	**27**	**0.27**	12	0.12	0	0		
3435CT	15	0.15	**32**	**0.32**	4	0.04	0.55	0.26
3435TT	1	0.01	5	0.05	**4**	**0.04**		
	2677GG		*2677GT*		*2677TT*			
Block 3	N	f	N	f	N	f	D′	r^2
1236CC	**35**	**0.35**	8	0.08	0	0		
1236CT	14	0.14	**35**	**0.35**	0	0	0.76	0.45
1236TT	0	0	4	0.04	**4**	**0.04**		

The bold values show the probability of an individual having the same type of genotype variant from two SNPs.

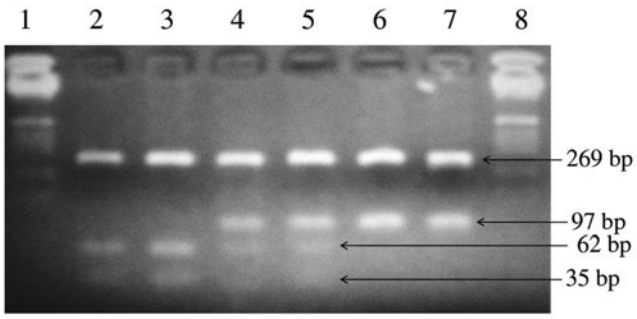
N, number of combinations; f, frequency of combinations; D′, linkage disequilibrium; r^2, correlation coefficient.

Discussion

Research on genes involved in drug absorption, distribution, metabolism, and excretion helps understand the pharmacokinetics and pharmacodynamics of drugs. The polymorphisms in the *MDR1* gene are increasingly explored today. Moreover, several studies have shown the importance of the three most common SNPs of the *MDR1* gene in response to drugs (Ling-Na *et al.*, 2011; Yan *et al.*, 2011; Munshi, 2012; Vivona *et al.*, 2012) and their involvement in the development of certain diseases (Cizmarikova *et al.*, 2010; Sabahi *et al.*, 2010; Khedri *et al.*, 2011). The 2677G > T/A polymorphism in exon 21 leading to amino acid change was the first SNP described, this SNP changes the amino acid (Ala893Ser/Thr) (Mickley *et al.*, 1998). Two years after Hoffmeyer *et al.* (2000) have described systematically 15 SNPs, including a silent SNP (3435C > T) in exon 26, which was found to influence protein expression and digoxin transportation. Currently, the number of SNPs identified in the *MDR1* gene is more than 50 and 3 insertions/deletions (Brinkmann *et al.*, 2001; Saito *et al.*, 2002; Evans and McLeod, 2003; Schwab *et al.*, 2003).

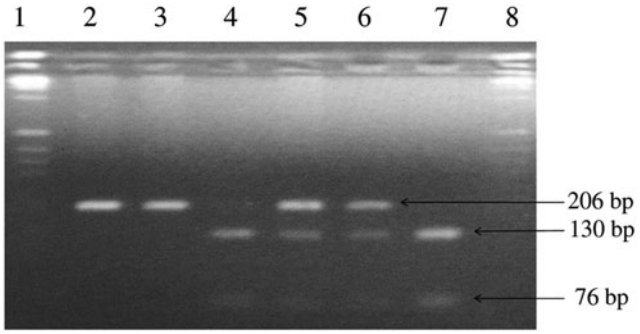
In the present study, the frequency of the wild-type 1236CC in exon 12 is greater than that observed among Caucasians and Asians, but less than that observed among Ghanaians. However, the frequency of 1236CT is comparable with that observed in other countries outside Serbia and Ghana. The frequency of the homozygous mutant 1236TT in our population is less than that observed among Caucasians and Asians except Ghanaians (Table 4). Even though this SNP (Gly412Gly) is a silent mutation, the 1236TT genotype has been linked with a reduced clearance of docetaxel (Bosch *et al.*, 2006) and also in early response to prednisone (Wasilewska *et al.*, 2007). It has been shown that patients with chronic myeloid leukemia carrying the 1236TT genotype respond better to imatinib (Dulucq *et al.*, 2008). However, this silent mutation in some cases has no impact in drug metabolism as demonstrated by Zhang *et al.*, (2008) in valacyclovir uptake. The results obtained in this study could contribute to identify the good responders and furthermore to predict the patients response to these drugs.

In exon 21, the frequency of the 2677GG genotype is higher than that observed in Caucasians and Asians, but lower compared with Ghanaians and African-Americans, while the frequency of heterozygous 2677GT is similar to



Lane 1, 8: 0.07- 12.2 kbp
Lane 2, 3: CC (homozygous)
Lane 4, 5: CT (heterozygous)
Lane 6, 7: TT (homozygous)

FIG. 2. Different profiles of *MDR1* gene polymorphism in exon 12 (C1236T) on a 3% agarose gel.

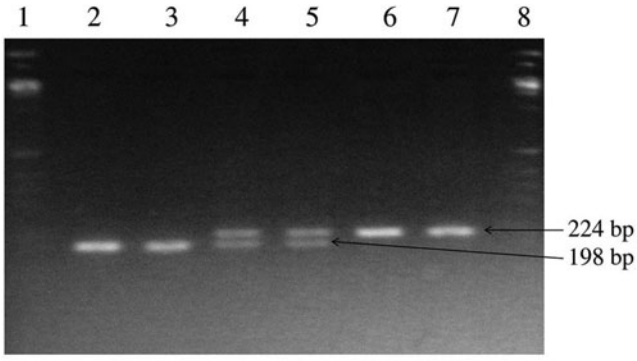


Lane 1, 8: 0.07- 12.2 kbp
Lane 2, 3: TT (homozygous)
Lane 4, 7: CC (homozygous)
Lane 5, 6: CT (heterozygous)

FIG. 4. Different profiles of *MDR1* gene polymorphism in exon 26 (C3435T) on a 3% agarose gel.

that of Caucasians, Saudis, and Turks. However, the frequency of 2677TT remains significantly lower than that observed in Caucasians, Asians, and Saudis. We have not found any variant of A allele in the locus 2677, the failure to detect this variant could be explained by our sample size (100) and by the fact that this SNP seems to have a geographical distribution as it is the case in the Ghanaian population and African-Americans who are from Africa (Table 4). Pan *et al.*, (2008) have noticed that patients with nonsmall cell lung cancer harboring the 2677GG genotype respond better to chemotherapy compared to other groups; a similar finding was obtained in patients with small cell lung cancer (Sohn *et al.*, 2006). Therefore, the identification of this SNP, which leads to amino acid exchange, might be helpful in the classification of our patients with small or nonsmall lung cancer before chemotherapy.

In exon 26, the frequency of the wild-type 3435CC is higher than that observed in Caucasians and Asians, similar to that in Saudis and Egyptians, outstandingly lower than that observed in Ghanaians. The heterozygous (3435CT) frequency is similar to that observed in Egyptians, Asians, and Caucasians, except Indians, but higher to that observed in Ghanaians. The frequency of homozygous variant type (3435TT) is close to that observed in the Egyptian population, lower than that observed in Caucasians and Asians, but higher than that observed in the Ghanaian population (Table 4). The overdominance of the 3435CC genotype is reported in African people (Ghanaians, Kenyans), and it is supposed to be a positive consequence of natural selection protecting against gastrointestinal tract infections (Mickley *et al.*, 1998; Schaeffeler *et al.*, 2001). It is reported that the 3435CC genotype is associated with a better response to Vinorelbine-based chemotherapy in nonsmall lung cancer patients (Pan *et al.*, 2008). As seen in our results, 39% of our population with nonsmall lung cancer could respond better than the other variants.



Lane 1, 8: 0.07- 12.2 kbp
Lane 2, 3: GG (homozygous)
Lane 4, 5: GT (heterozygous)
Lane 6, 7: TT (homozygous)

FIG. 3. Different profiles of *MDR1* gene polymorphism in exon 21 (G2677T) on a 3% agarose gel.

Several studies have found LD between C1236T, G2677T, and C3435T polymorphisms in the *MDR1* gene (Hoffmeyer *et al.*, 2000; Kim *et al.*, 2001; Allabi *et al.*, 2005; Zhang *et al.*, 2008; Al-Mohizea *et al.*, 2012). Indeed, our data show similar results with a moderate LD. It was shown that clustering *MDR1* SNPs into haplotypes will contribute to a better estimation of the functional value of the *MDR1* gene at a clinical level and understanding the contradictory studies on *MDR1* gene expression (Kim *et al.*, 2001). Thus, several studies have explored the clinical impact of the *MDR1* gene haplotypes. Potocnik *et al.*, (2008) have reported that patients harboring both the 1236TT and 2677TT genotypes were linked to a higher microsatellite instability in colorectal cancer compared to controls; as shown in Table 3, 4% of our population carry this haplotype. Therefore, screening this haplotype in our patients with colorectal cancer may help to identify the high microsatellite instability risk group. As shown in figure 1, the 1236C-2677G-3435C (CGC) haplotype in our study represents the most common with 53%. The frequency of this haplotype is higher than that observed in Caucasians, Asians, and Saudis, but lower than those observed in Ghanaians and African-Americans. Dulucq *et al.*, (2008) have found that the CGC haplotype is associated with a poor molecular response to imatinib in chronic myeloid leukemia patients. Hence, the

Table 4. Genotypic Distribution in Percentage of C1236T, C3435T, and G2677T/A in Different Populations

		C1236T			C3435T			G2677T/A						
Population	*N*	*CC*	*CT*	*TT*	*CC*	*CT*	*TT*	*GG*	*GT*	*GA*	*TT*	*TA*	*AA*	*Reference*
Moroccans	100	43	49	8	39	51	10	49	47	0	4	0	0	This study
Caucasians														
Portuguese	100	Na	Na	Na	12	47	41	31	43	Na	26	Na	Na	Cavaco *et al.* (2003)
Serbians	158	23	61	16	19	54	27	26	52	3	15	4	0	Milojkovic *et al.* (2011)
Romanians	465	20.7	47.1	21.2	26.7	50.3	23	26.9	49	2.37	20.2	1.30	0.22	Sipeky *et al.* (2011)
Czechs	189	31.7	47.1	21.2	21.2	44.9	33.9	29.6	47.1	1.1	22.2	0	0	Pechandová *et al.* (2006)
Polish	204	Na	Na	Na	22	51	27	38.7	39.7	4	17.6	2	4	Kurzawski *et al.* (2006)
Germans	461	34.4	49.2	16.4	21	50	29	31	31	2	16	2	0	Cascorbi *et al.* (2001)
Russians	290	Na	Na	Na	21.4	48.6	30	30.3	44.9	4.1	18.3	2.4	0	Gaikovitch *et al.* (2003)
Spanish	204	Na	Na	Na	27	51	22	Na	Na	Na	Na	Na	Na	Vicente *et al.* (2008)
Indians	96;97;101	15.6	46.9	37.5	24.7	41.2	34	13.9	47.5	25.7	25.7	8.9	0	Lakhan *et al.* (2009)
Asians														
Chinese	200	10.5	47.5	42	30	53.5	16.5	17.5	37.5	11	21	10.5	2.5	Zhang *et al.* (2008)
Japanese	154	11	46.8	42.2	35.7	47.4	16.9	19.5	31.8	14.9	18.2	13	2.6	Omoto *et al.* (2006)
Africans														
A-Americans	5	Na	Na	Na	Na	Na	Na	100	0	0	0	0	0	Zheng *et al.* (2002)
Ghanaians	194	100	0	0	84	19	2	100	0	0	0	0	0	Kudzi *et al.* (2010)
Kenyans	80	Na	Na	Na	70	26	4	Na	Na	Na	Na	Na	Na	Hamdy *et al.* (2003)
Sudanese	51	Na	Na	Na	52	43	6	Na	Na	Na	Na	Na	Na	Hamdy *et al.* (2003)
Others														
Saudis	189;179;184	38.6	35.4	26	35.2	45.2	19.6	38	41.3	0.5	19.1	1.1	0	Al-Mohizea *et al.* (2012)
Egyptians	200	Na	Na	Na	34	51.5	14.5	Na	Na	Na	Na	Na	Na	Hamdy *et al.* (2003)
Turks	107	20.6	50.5	28.9	27.1	41.1	31.8	21.5	44.9	3.7	22.4	7.5	0	Gümüş-Akay *et al.* (2010)
Iranians	131	Na	Na	Na	17.6	57.2	35.4	Na	Na	Na	Na	Na	Na	Sabahi *et al.* (2010)

N, number of samples tested; Na, not assessed.

identification of this haplotype in our patients undergoing imatinib treatment will help predict the response to imatinib. The second most common haplotype represented by the 1236T-2677T-3435T (TTT) 21% is lower than those observed in Caucasians, Asians, and Saudis, but higher than observed among Ghanaians and African-Americans (Kim *et al.*, 2001; Tang *et al.*, 2002., Kudzi *et al.*, 2010; Al-Mohizea *et al.*, 2012). Woillard *et al.*, (2010) have reported that patients who have benefited from grafts from a donor carrying the TTT haplotype are significantly exposed to graft loss compared to patients who have received their grafts from donors carrying the CGC haplotype. Therefore, the haplotypes determination of the *MDR1* gene could facilitate the identification of suitable donors for patients undergoing renal transplant.

Our results suggest that the Moroccan population retains an intermediate position in the distribution of the 3 SNPs. This fact could be explained by the ethnic composition (Arabs and Berbers) and the historical influence of sub-Saharan Africa through the trans-Saharan commercial trade (Abitbol, 1980) with possible gene flow. Therefore, other studies are needed to establish the functional impact of these SNPs and haplotypes on the pharmacokinetics of drugs that are substrates of the *MDR1* gene in Moroccan patients.

Conclusion

We have established the allelic, genotypic, and haplotypic frequencies of three major polymorphisms of the *MDR1* gene from a sample of the Moroccan population. Overall, we have found that the distribution of the C1236T, G2677T, and C3435T SNPs conserves an intermediate position compared to Caucasians, Asians, and Sub-Saharan Africans. This determination of functional polymorphisms of the *MDR1* gene in our healthy population could be a starting point to facilitate future research on pharmacokinetics and pharmacodynamics of drugs that are *MDR1* substrates.

Acknowledgments

We authors thank the Hassan II Academy of Science and Technology for providing us with financial support for this study.

Disclosure Statement

No competing financial interests exist.

References

Abitbol, M. (1980). Le Maroc et le commerce transsaharien du XVIIe au début du XIXe siècle. Revue de l'Occident Musulman et de la Méditerranée **30,** 5–19.

Allabi, A.C., Horsmans, Y., Issaoui, B., and Jean-Luc, G. (2005). Single nucleotide polymorphisms of ABCB1 (MDR1) gene and distinct haplotype profile in a West Black African population. Eur J Clin Pharmacol **61,** 97–102.

Al-Mohizea, A.M., Alkharfy, K.M., Bagulb, K.M., Alghamdi, A.M., Al-Jenoobi, F.I., Al-Muhsen, S., Halwani, R., *et al.* (2012). Genetic variability and haplotype profile of MDR1 in Saudi Arabian males. Mol Biol Rep **39,** 10293–10301.

Bosch, T.M., Huitema, A.D.R., Doodeman, V.D., Jansen, R., Witteveen, E., Smit, W.M., Jansen, R.L., *et al.* (2006). Pharmacogenetic screening of CYP3A and ABCB1 in relation to population pharmacokinetics of docetaxel. Clin Cancer Res **12,** 5786–5793.

Brinkmann, U., Roots, I., and Eichelbaum, M. (2001). Pharmacogenetics of the human drug-transporter gene MDR1: impact of polymorphisms on pharmacotherapy. Drug Discov Today **6,** 835–839.

Cascorbi, I., Gerloff, T., Johne, a, Meisel, C., Hoffmeyer, S., Schwab, M., Schaeffeler, E., *et al.* (2001). Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther **69,** 169–174.

Cavaco, I., Gil, J.P., Gil-Berglund, E., and Ribeiro, V. (2003). CYP3A4 and MDR1 alleles in a Portuguese population. Clin Chem Lab Med **41,** 1345–1350.

Cizmarikova, M., Wagnerova, M., Schonova, L., Habalova, V., Kohut, A., Linkova, A., Sarissky, M., *et al.* (2010). MDR1 (C3435T) polymorphism: relation to the risk of breast cancer and therapeutic. Pharmacogenet Genomics J **10,** 62–69.

Dulucq, S., Bouchet, S., Turcq, B., Lippert, E., Etienne, G., Reiffers, J., Molimard, M., *et al.* (2008). Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood **112,** 2024–2027.

Ekhart, C., Rodenhuis, S., Smits, P.H.M., Beijnen, J.H., and Huitema, A.D.R. (2009). An overview of the relations between polymorphisms in drug metabolising enzymes and drug transporters and survival after cancer drug treatment. Cancer Treat Rev **35,** 18–31.

Evans, W.E., and McLeod, H.L. (2003). Pharmacogenomics—drug disposition, drug targets, and side effects. New Engl J Med **348,** 538–549.

Fojo, A.T., Ueda, K., Slamon, D.J., Poplack, D.G., Gottesman, M.M., and Pastan, I. (1987). Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci U S A **84,** 265–269.

Fromm, M.F. (2002). Genetically determined differences in P-glycoprotein function: implications for disease risk. Toxicology **181,** 299–303.

Gaikovitch, E. A., Cascorbi, I., Mrozikiewicz, P. M., Brockmöller, J., Frötschl, R., Köpke, K., Gerloff, T., *et al.* (2003). Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. Eur J Clin Pharmacol **59**, 303–312.

Gümüş-Akay, G., Rüstemoğlu, A., Karadağ, A., and Sunguroğlu, A. (2010). Haplotype-based analysis of MDR1/ABCB1 gene polymorphisms in a Turkish population. DNA Cell Biol **29,** 83–90.

Hamdy, S. I., Hiratsuka, M., Narahara, K., Endo, N., El-Enany, M., Moursi, N., Ahmed, M. S.-E., *et al.* (2003). Genotype and allele frequencies of TPMT, NAT2, GST, SULT1A1 and MDR-1 in the Egyptian population. Br J Clin Pharmacol **55**, 560–569.

Hoffmeyer, S., Burk, O., Von Richter, O., Arnold, H.P., Brockmöller, J., Johne, A., Cascorbi, I., *et al.* (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. Proc Natl Acad Sci USA **97,** 3473–3478.

Ishikawa, T., Hirano, H., Onishi, Y., Sakurai, A., and Tarui, S. (2004). Functional evaluation of ABCB1 (P-glycoprotein) polymorphisms: high-speed screening and structure-activity relationship analyses. Drug Metab Pharmacokinet **19,** 1–14.

Khedri, A., Nejat-Shokouhi, A., Salek, R., Esmaeili, H., Mokhtarifar, A., Entezari Heravi, R., Tatari, F., *et al.* (2011). Association of the colorectal cancer and MDR1 gene polymorphism in an Iranian population. Mol Biol Rep **38,** 2939–2943.

Kim, D.H., Park, J.Y., Sohn, S.K., Lee, N.Y., Baek, J.H., Jeon, S.B., Kim, J.G., *et al.* (2006). Multidrug resistance-1 gene polymorphisms associated with treatment outcomes in *de novo* acute myeloid leukemia. Int J Cancer **118,** 2195–2201.

Kim, R., Leake, B., Choo, E., Dresser, G., Kubba, S., Schwarz, U., Taylor, A., *et al.* (2001). Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther **70,** 189–199.

Kudzi, W., Dodoo, A.N., and and Mills, J.J. (2010). Genetic polymorphisms in MDR1, CYP3A4 and CYP3A5 genes in a Ghanaian population: a plausible explanation for altered metabolism of ivermectin in humans? BMC Med Genet **11,** 1–8.

Kurzawski, M., Pawlik, A., Górnik, W., and Drodzik, M. (2006). Frequency of common MDR1 gene variants in a Polish population. Pharmacological reports **58**, 35–40.

Lakhan, R., Misra, U., Kalita, J., Pradhan, S., Gogtay, N., Singh, M., and Mittal, B. (2009). No association of ABCB1 polymorphisms with drug-refractory epilepsy in a north Indian population. Epilepsy Behav: E&B **14**, 78–82.

Ling-Na, N., Jian-Yong, L., Kou-Rong, M., Chun, Q., Su-Jiang, Z., Hai-Rong, Q., and Si-Xuan, Q. (2011). Multidrug resistance gene (MDR1) polymorphisms correlate with imatinib response in chronic myeloid leukemia. Med Oncol **28,** 265–269.

Marzolini, C., Paus, E., Buclin, T., and Kim, R. (2004). Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther **75,** 13–33.

Mickley, L.A., Lee, J.S., Weng, Z., Zhan, Z., Alvarez, M., Wilson, W., Bates, S.E., *et al.* (1998). Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. Blood **91,** 1749–1756.

Miller, S., Dykes, D., and Polesky, H. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res **16,** 1215.

Milojkovic, M., Stojnev, S., Jovanovic, I., Ljubisavljevic, S., Stefanovic, V., and Sunder-Plassman, R. (2011). Frequency of the C1236T, G2677T/A and C3435T MDR1 gene polymorphisms in the Serbian population. Pharmacological reports: PR **63**, 808–814.

Munshi, A. (2012). Genetic variation in MDR1, LPL and eNOS genes and the response to atorvastatin treatment in ischemic stroke. Hum Genet **131,** 1775–1781.

Omoto, C.K., Akamura, T.N., Akaeda, T.S., Roetz, D.L.K., Amada, T.Y., Matsu, H.O., Oyama, T.K., *et al*. (2006). Regular article MDR1 haplotype frequencies in Japanese and Caucasian, and in Japanese patients with colorectal cancer and esophageal cancer. Drug Metab Pharmacokinet **21**, 126–132.

Pan, J., Han, J., Wu, J., Sheng, L., Huang, H., and Yu, Q. (2008). MDR1 single nucleotide polymorphisms predict response to vinorelbine-based chemotherapy in patients with non-small cell lung cancer. Respiration **75,** 380–385.

Pechandová, K., Buzková, H., Slanar, O., and Perlík, F. (2006). Polymorphisms of the MDR1 gene in the Czech population. Folia Biol **52**, 184–189.

Potocnik, U., Glavac, D., and Dean, M. (2008). Common germline MDR1/ABCB1 functional polymorphisms and haplotypes modify susceptibility to colorectal cancers with high microsatellite instability. Cancer Genet Cytogenet **183,** 28–34.

Sabahi, Z., Salek, R., Heravi, R., Mosaffa, F., Avanaki, Z., and Behravan, J. (2010). Association of gastric cancer incidence with MDR1 gene polymorphism in an ethnic Iranian population. Indian J Cancer **47,** 317–321.

Saito, S., Iida, A., Sekine, A., Miura, Y., Ogawa, C., Kawauchi, S., Higuchi, S., *et al.* (2002). Three hundred twenty-six genetic

variations in genes encoding nine members of ATP-binding cassette, subfamily B (ABCB/MDR/TAP), in the Japanese population. J Hum Genet **47,** 38–50.

Schaeffeler, E., Eichelbaum, M., Brinkmann, U., Penger, A., Asante-poku, S., Zanger, U.M., and Schwab, M. (2001). Frequency of C3435T polymorphism of MDR1 gene in African people Prediction of severe disseminated adenovirus infection by serum PCR. Lancet **358,** 383–384.

Schwab, M., Eichelbaum, M., and Fromm, M.F. (2003). Genetic polymorphisms of the human MDR1 drug transporter. Ann Rev Pharmacol Toxicol **43,** 285–307.

Shen, D.W., Fojo, A., Chin, J.E., Roninson, I.B., Richert, N., Pastan, I., and Gottesman, M.M. (1986). Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification. Science **232,** 643–645.

Shon, J.-H., Yoon, Y.-R., Hong, W.-S., Nguyen, P.M., Lee, S.-S., Choi, Y.-G., Cha, I.-J., *et al.* (2005). Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism. Clin Pharmacol Ther **78,** 191–201.

Sipeky, C., Csongei, V., Jaromi, L., Safrany, E., Maasz, A., Takacs, I., Beres, J., *et al.* (2011). Genetic Variability and Haplotype Profile of MDR1 (ABCB1) in Roma and Hungarian Population Samples with a Review of the Literature. Drug Metab Pharmacokinet **26**, 206–215.

Sohn, J.W., Lee, S.Y., Lee, S.J., Kim, E.J., Cha, S.I., Kim, C.H., Lee, J.-T., *et al.* (2006). MDR1 polymorphisms predict the response to etoposide-cisplatin combination chemotherapy in small cell lung cancer. Jpn J Clin Oncol **36,** 137–141.

Tanabe, M., Ieiri, I., Nagata, N., Inoue, K., Ito, S., Kanamori, Y., Takahashi, M., *et al.* (2001). Expression of P-gly-coprotein in human placenta: relation to genetic poly-morphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther **297,** 1137–1143.

Tang, K., Ngoi, S., Gwee, P., Chua, J.Z., Lee, E.J., Chong, S.S., and Lee, C.G. (2002). Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. Pharmacogenetics **12,** 437–450.

Ueda, K., Pastan, I., and Ottesman, M.M. (1987). Isolation and sequence otfh e promoter region of the human multidrug-resistance (P-glycoprotein) gene. J Biol Chem **262,** 17432–17436.

Vicente, J., Sinues, B., Fanlo, A, Vasquez, P., Medina, J. C., and Martinez-Jarreta, B. (2008). Polymorphism C3435T of the MDR1 gene in Central Americans and Spaniards. Mol Biol Rep **35**, 473–478.

Vivona, D., Bueno, C.T., Lima, L.T., Hirata, R.D.C., Hirata, M.H., Luchessi, A.D., Zanichelli, M.A., *et al.* (2012). ABCB1 haplotype is associated with major molecular response in chronic myeloid leukemia patients treated with standard-dose of imatinib. Blood Cells Mol Dis **48,** 132–136.

Wang, D., Johnson, A., Papp, A., Kroetz, D., and Sadée, W. (2005). Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics **15,** 693–704.

Wasilewska, A., Zalewski, G., Chyczewski, L., and Zoch-Zwierz, W. (2007). MDR-1 gene polymorphisms and clinical course of steroid-responsive nephrotic syndrome in children. Pediatr Nephrol **22,** 44–51.

Woillard, J., Rerolle, J., Picard, N., Rousseau, A., Guillaudeau, A., Munteanu, E., Essig, M., *et al.* (2010). Donor P-gp polymorphisms strongly influence renal function and graft loss in a cohort of renal transplant recipients on cyclosporine therapy in a long-term follow-. Clin Pharmacol Ther **88,** 95–100.

Yan, P., Huang, X., Yan, F., Xu, L., and Jiang, Y. (2011). Influence of MDR1 gene codon 3435 polymorphisms on outcome of platinum-based chemotherapy for advanced non small cell lung cancer. Asian Pac J Cancer Prev **12,** 2291–2294.

Yoo, J., Lee, Y., Kim, Y., Rha, S.Y., and Kim, Y. (2008). SNPAnalyzer 2.0: a web-based integrated workbench for linkage disequilibrium analysis and association analysis. BMC Bioinform **9,** 1–10.

Zhang, Y., Jiang, X.-H., Hu, Y.-Q., Li, Z.-R., Su, L., Wang, Z.-G., and Ma, G. (2008). 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 **66,** 247–254.

Zheng, H., Webber, S., Zeevi, A., Schuetz, E., Zhang, J., Lamba, J., Bowman, P., *et al.* (2002). The MDR1 polymorphisms at exons 21 and 26 predict steroid weaning in pediatric heart transplant patients. Human Immunol **63**, 765–770.

Address correspondence to:
Sellama Nadifi, MD, PhD
Genetics and Molecular Pathology Laboratory
Medical School of Casablanca
University Hassan II
19 Rue Tarik Ibnou Ziad
Casablanca BP. 9154
Morocco

E-mail: nadifi@labgenmed.com

Received for publication May 18, 2013; received in revised form July 1, 2013; accepted July 1, 2013.