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Genotype and Allele Frequencies of MDR-1 in Bahrain Population

معدل تكرار الطراز الجيني والأليل لجين م MDR-1 في الشعب البحريني

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Abstract: The goal of this study was to determine the frequency of the single nucleotide polymorphism C3435T in MDR1 gene in the Bahraini population, and to compare them with the frequencies established in other ethnic populations. Genotyping was carried out on 184 unrelated Bahraini subjects. A PCR-RFLP assay was applied for the determination of *MDR-1* variants. Results showed that 64 (34.8%) of the studied Bahraini subjects were homozygous for the *CC* genotype, 84 (45.7%) were heterozygous for the *CT* genotype and 36 (19.5%) were homozygous for the *TT* genotype. The frequencies of the wild-type allele *3435C* and the *3435T* variant in the *MDR-1* Gene were found to be 0.58 and 0.42, respectively. In conclusion, Bahrainis resemble other Arabs with regard to allelic frequencies of the *MDR-1* variants. The results of MDR-1 genotyping in Bahraini individuals may provide a framework for more rational use of drugs that are substrates for *MDR-1*. The data obtained may be useful in clinical pharmacogenetic investigations and epidemiological studies of the *MDR-1* gene variation.
Keywords: *MDR-1 Gene, Human Population Genetics, Bahrain.*

المستخلص: الهدف من الدراسة هو تحديد مدى تكرار النمط الجيني الوراثي (ت 3435 س) لجين م در-1 في الشعب البحريني ومقارنتها بالشعوب الأخرى.أجريت الدراسة علي 184 شخصا من البحرينين باستخدام طريقة التفاعل البلمري المتسلسل مع استخدام الأنزيمات القاطعة للحامض النووي.أظهرت نتائج الدراسة أن 34.5% من العينة التي أجريت عليها الدراسة كانت تحمل الطراز الجيني المتجانس (س س) وأن 45.7% من العينة كانت تحمل الطراز الهجين (س ت) بينما يحمل 19.5% من العينة الطراز الجيني المتجانس (ت ت). وأن معدل تكرار الأليل 3435 س والأليل 3435 ت كان 0.58 و0.42 علي التوالي.أظهرت الدراسة أن الشعب البحريني يماثل نظرائه من الشعوب العربية بالنسبة لمعدل تكرار الأليلات الخاصة بجين م د ر-1. كما توفر هذه الدراسة إطارا للاستخدام الهادف للعقاقير الطبية التي تستخدم جين م د ر-1 في الشعب البحريني. وهي كذلك مفيدة في الأبحاث الوراثية للعقاقير ودراسات الأوبئة للأشكال المختلفة لجين م د ر-1.
كلمات مدخلية : *م در-1 جين ، علم الوراثة في الشعوب الإنسانية ، البحرين.*

INTRODUCTION

The response to the medications and the adverse effects of the drugs varies between individuals in the same population. Individual genetic differences and genetic polymorphisms of the drug metabolizing enzymes play an important role on the metabolism and distribution of the drugs (Ambudkar, *et al.* 1999).

A key pharmacological barrier limiting

the accessibility of drugs to various target cells and tissues is P-glycoprotein (P-gp), also known as ABCB1 or MDR1. P-gp is a 170-kD plasma glycoprotein that was discovered by Juliano and Ling in multidrug-resistance (MDR) cancer cells (Juliano and Ling, 1976). In addition to being highly expressed in tumor cells, P-gp is also expressed in the liver, kidney, pancreas, small intestine and colon, adrenal gland, brain and testes (Schinkel, *et al.* 1994; Silverman and Schrenk, 1992; Cordon-Cardo, *et al.* 1990; Thiebaut, *et al.* 1987).

P-gp is an ATP-dependent efflux pump that plays an important role in the bioavailability of a wide variety of drugs, including the cardiac glycoside digoxin, anthracycline antibiotics, vinblastine, daunomycin, and cyclosporin A (Gottesman, *et al.* 1995).

In humans, there are two MDR genes, *MDR1* and *MDR2*. The *MDR1* gene is located on the long arm of chromosome 7 and consists of a core promoter region and 28 exons ranging in size from 49 to 209 bp (Ambudkar, *et al.* 2003). *MDR1* encodes P-gp, whereas *MDR2* encodes a P-gp specific for phosphatidylcholine translocation in cells (Gottesman and Pastan, 1993). Over 50 single nucleotide polymorphisms (SNPs) have been reported for *MDR1* gene (http://www.ncbi.nlm.nih.gov/SNP/GeneGt.cgi?geneID=5243), of which more than 20 are known to be silent (Komar, 2007).

Ethnic differences in the response to drugs have been related to genotypic variants (polymorphisms) of key enzymes and proteins that affect the safety and efficacy of a drug in the individual patient (Evans and Relling, 1999).

Recently, a functional single nucleotide polymorphism (SNP) resulting in a C to T transition in exon 26 (C3435T) were identified, where the homozygous T allele was associated with more than two-fold lower duodenal P-gp expression levels, compared to CC genotypes (Hoffmeyer, *et al.* 2000).

The frequencies of the *MDR-1* alleles have been studied in many ethnic groups, and demonstrated striking interethnic variation in their distribution. The frequency of the homozygous CC genotype was highest among Africans and lowest in the South-west Asians (Ameyaw, *et al.* 2001). Heterozygous individuals displayed an intermediate phenotype (Balram, *et al.* 2003). However, no information is available for Bahraini population. The present study investigated for the first time the frequencies of the *MDR-1* (C3435T) polymorphisms in 184 Bahraini individuals, providing a basis for future clinical studies concerning variability in the bioavailability to drugs known to be substrates for MDR-1.

MATERIALS AND METHODS

Study Populations

The samples studied were collected from 184 unrelated Bahraini individuals under institutionally approved internal review board protocols, with informed consent. Genomic DNA was prepared from whole blood by standard methods (Sambrook, *et al.* 1989).

Genotyping

DNA samples were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Hamdy, *et al.* 2003). A 231-bp fragment containing nucleotide 3435 was amplified with the following primers: MDR-1F (5'- ACTCTT GTTTTCAGCTGCTTG-3') and MDR-1R (5'-AGAGACTTACATTAGGCAGTGACTC-3'). PCR amplification consisted of an initial denaturation step at 94°C for 5 min followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. The final extension step was performed at 72°C for 10 min.

The PCR products were digested with *Bfu CI* (New England *Biolabs*) at 37°C overnight. The digested products were separated on a 3% agarose gel. The wild-type C allele, but not the mutant T allele contains a *Bfu CI* restriction site, thereby allowing discrimination of the digested products. Digestion of the 231-bp fragment by *Bfu CI* yielded two fragments of 163 and 68 bp. All subjects were genotyped twice for all the tested mutations, which makes the possibility of genotyping error less likely.

Statistical Analysis

Statistical analysis was performed using SPSS statistical package for Windows (version

15). Allele and genotype frequencies were calculated by direct counting; the Hardy-Weinberg equilibrium was assessed by an exact test (Guo and Thompson, 1992) provided by the Arlequin program (Excoffier, *et al.* 2005).

The frequency of each allele in our subjects is given together with the 95% confidence interval (CI). Differences in allele frequencies between Bahrainis and other ethnic populations were measured using Chi square test and Fisher's exact test. A *P*-value <0.05 was considered statistically significant.

RESULTS

The genotype frequencies obtained from all individuals are summarized in Table 1. Genotyping of the Bahraini subjects for the C3435T mutation revealed a genotype distribution of (*C/C, C/T, and T/T*: 34.78%, 45.65%, 19.57%), respectively. The frequencies of the wild-type allele (*3435C*) and the *3435T* variant were found to be 0.576 (95% CI: 0.524 - 0.627) and 0.424 (95% CI: 0.373 - 0.476), respectively (Table 1, Figure 1).

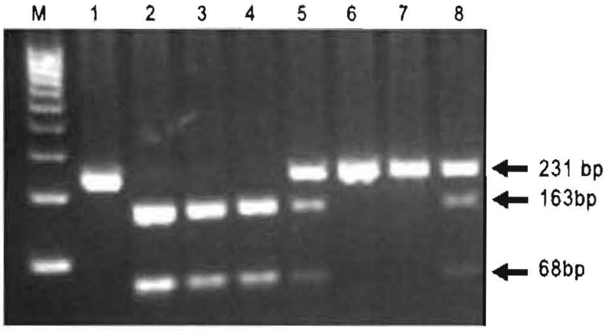

Fig. 1. Electrophoresis Patterns for MDR1 Genotypes by PCR-RFLP Based Assay. M is a 100 bp DNA marker. Samples 1, 6 and 7 are homozygous CC genotype which lack Bfu CI restriction site. Samples 2, 3 and 4 are homozygous TT genotype which has Bfu CI restriction site. Sample 5 and 8 are heterozygous CT genotype.

These results (Table 1) were in good accordance with the expected genotype distributions of the tested genes, calculated by the Hardy–Weinberg law (p > 0.05, Chi squared goodness of fit). The frequencies of the tested alleles in the Bahraini subjects, compared with data reported for other ethnic populations are given in Table (2).

DISCUSSION

Bahrain is a group of islands located in the Arabian Gulf with a population less than 1,000,000 (Al-Hadithi, 1993). Bahrain location in the Arabian Gulf has brought rule and influence from the Sumerians, Assyrians, Babylonians, Persians, and finally the Arabs.

The Bahraini population is an Arab peninsula population characterized by genetic homogeneity due to the tribal structure of their society, large family size and extensive consanguinity.

The tribal structure means that large numbers of individuals are descended from a limited number of founder members. In addition, the high frequency of consanguineous marriages leads to an increase in the prevalence of homozygosity (De Costa, *et al.* 2002; Al-Hadithi, 1993).

Polymorphisms in the human genome contribute to wide variations in how individuals respond to medications. Analysis of the sequence of the human genome has shown that the extent of genetic variation in humans is greater than what had been estimated, and the most common sequence variation is the SNP (Sauna, *et al.* 2007). A number of SNPs in the *MDR-1* gene have been identified as clinically important for drug response, which increases the need for genotyping of these SNPs in different population for individualizing drug treatment (Kimchi-Sarfaty, *et al.* 2007a). Although P-gp expression is highly

Table 1. The Genotype, Allele Frequencies, and Heterozygosities of the MDR1 Gene among 184 Bahraini Subjects

Population	N	MDR1 Genotype	Number Observed (and Percentage)	MDR1 Allele Frequency		Heterozygosity	
				C	T	Observed	Expected
Bahraini	184	CC	64 (34.8)	0.576	0.424	0.457	0.490
		CT	84 (45.7)				
		TT	36 (19.5)				

Table 2. Genotype Percentages and Allele Frequencies of MDR-1 Polymorphism in Various Ethnic Groups.

Population samples (n)	Genotype Percentage			Allele Frequency		P (vs. Bahraini)	Reference
	C/C	C/T	T/T	C	T		
Bahraini (184)	34.8	45.7	19.6	0.576	0.424		This study
Egyptians (200)	34	51.5	14.5	0.600	0.400	NS[a]	Hamdy, *et al.* 2003
Saudi (96)	37	38	26	0.550	0.450	NS[a]	Ameyaw, *et al.* 2001
Iranian (200)	20	52.5	27.5	0.463	0.537	<0.0001[a]	Azarpira and Aghdaie 2006
Turkish (150)	20	53	27	0.470	0.530	<0.0001[a]	Turgut, *et al.* 2006
British (190)	24	48	28	0.480	0.520	<0.0001[a]	Ameyaw, *et al.* 2001
Portuguese (100)	22	42	36	0.430	0.570	<0.0001[a]	Ameyaw, *et al.* 2001
German (188)	28	48	24	0.520	0.480	0.0134[a]	Hoffmeyer, *et al.* 2000
French (81)	36	42	22	0.570	0.430	NS[a]	Anglicheau, *et al.* 2003
Polish (122)	42	41	16	0.620	0.380	NS[a]	Jamroziak, *et al.* 2002
Ashkenazi Jews (100)	42	46	12	0.650	0.350	0.0008[a]	Ostrovsky, *et al.* 2004
Japanese (50)	34	46	20	0.570	0.430	NS[a]	Schaeffeler, *et al.* 2001
Japanese (154)	35.7	47.4	16.9	0.594	0.406	NS[a]	Komoto, *et al.* 2006
Chinese (132)	32	42	26	0.530	0.470	NS[a]	Ameyaw, *et al.* 2001
Indians (93)	18	39	43	0.380	0.620	<0.0001[a]	Balram, *et al.* 2003
Filipino (60)	38	42	20	0.590	0.410	NS[a]	Ameyaw, *et al.* 2001
South-west Asians (89)	15	38	47	0.340	0.660	<0.0001[a]	Ameyaw, *et al.* 2001
Sudanese (51)	52	43	6	0.730	0.270	<0.0001[b]	Ameyaw, *et al.* 2001
Kenyan (80)	70	26	4	0.830	0.170	<0.0001[b]	Ameyaw, *et al.* 2001
Ghanaian (206)	66	34	0	0.830	0.170	<0.0001[b]	Ameyaw, *et al.* 2001

Differences in allele frequencies were measured using Chi square test [a], and Fisher's exact test [b]. NS, There were no significant differences (p>0.05). (n) Number of subjects.

variable between subjects, the molecular basis for this variation was not clear until recently, with the description of functional (C3435T) SNP in exon 26 of the *MDR-1* gene (Hoffmeyer, *et al.* 2000). The silent mutation C3435T is the most commonly reported polymorphism linked to different responses of patients to various *MDR1* substrates. The frequency of this mutation is different significantly between different populations (Kimchi-Sarfaty, *et al.* 2007b).

The present study investigated for the first time the frequency of the C3435T SNP in *MDR1* gene among unrelated Bahraini subjects, and compared the results with data reported for other ethnic groups (see, table 2). The results obtained showed genotype frequencies of 34.8%, 45.7%, and 19.6% for *C/C*, *C/T* and *T/T* genotypes, respectively.

The frequencies of the wild *C*-allele (0.576) and the wild-genotype *C/C* (34.78%) in Bahraini subjects were not significantly different from the reported studies for Arab populations like Saudi Arabians (0.550 and 37%, Ameyaw, *et al.* 2001) and Egyptians (0.600 and 34%, Hamdy, *et al.* 2003), which may be attributed to the common Arab origin of these populations.

However, the frequency of the wild *C*-allele in Bahraini subjects was not significantly different from a number of European populations, including British, Portuguese, Germans (Ameyaw, *et al.* 2001), French (Anglicheau, *et al.* 2003) and Polish (Jamroziak, *et al.* 2002).

The frequency of the wild C-allele is significantly different from that reported for Ashkenazi Jews (Ostrovsky, *et al.* 2004), Turkish population (Turgut, *et al.* 2006), Iranians (Azarpira and Aghdaie, 2006) and Indians

(Balram, *et al.* 2003).

It was also different from the South-west Asians, Africans and African-Americans (Ameyaw, *et al.* 2001).

Interestingly, the frequency of the wild *C*-allele in Bahraini subjects did not differ from that reported in East-Asian populations including Japanese (Komoto, *et al.* 2006; Schaeffeler, *et al.* 2001), Chinese and Filipinos (Ameyaw, *et al.* 2001).

There was a significant relationship of the silent C3435T SNP with intestinal P-gp expression levels. Individuals who were homozygous for the 3435T variant had significantly decreased intestinal P-gp expression (Hoffmeyer, *et al.* 2000).

About 20% of Bahraini subjects were homozygous for the 3435T and 42% of them have at least one T allele, with lower P-gp level in small intestine. As P-gp has an important role in the bioavailability of a wide variety of drugs, including digoxin, anthracycline antibiotics, vinblastine, daunomycin, and cyclosporin A (Gottesman, *et al.* 1995). So, these drugs must be used cautiously in those subjects. Also, association studies between 3435T allele of MDR1 gene and these drugs in Bahraini population are needed.

The Europeans and Asians had lower frequencies of the C-allele compared to that of Africans. The high frequency of the C-allele in Africans implies overexpression of P-gp. As overexpression of P-gp has been associated with altered drug absorption, therapy resistant malignancies, this SNP may provide a useful approach to individualize therapy and it may have important therapeutic and prognostic implications for use of P-gp dependent drugs in individuals of African origin.

The association of MDR1 allele frequencies with certain drugs and diseases constitute a topic of intense research (Glas, *et al.* 2004; Brant , *et al.* 2003; Drozdzik, *et al.* 2003). Kim, *et al.* (2003) showed lack of association between C3435T SNP and multidrug resistant epilepsy. Also, Peles, *et al.* (2007) showed no association with therapeutic response to paroxetine in patients with major depressive disorder, while evidence is accumulating in favor of an association between MDR1 and altered risk of different diseases (Kimchi-Sarfaty, *et al.* 2007a; Lee and Bendayan, 2004).

The substrate specificity of P-gp is altered by synonymous and silent SNPs. It has been suggested that silent SNPs may affect protein translation rates hence influencing protein folding and activity. Therefore, silent SNPs that do not change the coding sequence of the protein may contribute to altered pharmacokinetics of substrate drugs and development of certain disease conditions (Kimchi-Sarfaty, *et al.* 2007a).

Analyzing the silent C3435T polymorphism is other Arab populations is needed to better understand its function in P-gp activity.

CONCLUSION

The results of *MDR-1* genotyping in Bahraini individuals may provide a framework for more rational use of drugs that are substrates for *MDR-1*. It should be noted that this was the first description of these alleles in Bahraini population which does not exclude the possibility of the presence of new SNPs unique to the Bahraini population.

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