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Association of *MDR1* C3435T and C1236T single nucleotide polymorphisms with male factor infertility

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ABSTRACT. Infertility affects 1 in 6 couples and approximately 1 in 25 men. Male factor infertility is a major cause of spermatogenic anomalies, the causes of which are largely unknown. Impaired reproductive functions in men might result from physiological, genetic, and/or environmental factors such as xenobiotics. The multi-drug resistance1 (*MDR1*) gene encodes a P-glycoprotein which has a role in the active transport of various substrates providing protection of somatic cells from potentially toxic substances, including xenobiotics. MDR1 is highly expressed at the luminal surface of capillary endothelial cells, and is expressed in Leydig cells, testicular macrophages, and Sertoli cells. We performed genotype and haplotype analyses of *MDR1* in 192 infertile and 102 fertile Turkish men for the genetic markers C1236T and C3435T, using polymerase chain

reaction-restriction fragment length polymorphism analysis. In the overall population, correlations were analyzed in all genotype models. We found that the C3435T polymorphism TT *vs* CT genotypes showed statistically significant differences in their association with infertility (P = 0.045), and that the CT genotype was associated with high sperm DNA damage (P = 0.02), suggesting that the CT genotype might be a susceptibility factor for infertility. Additionally, the T-T haplotype was significantly more frequent in the control group (13.2 *vs* 6.5%; odds ratio = 0.459, 95%CI = 0.259-0.814, P = 0.006). This study showed that MDR1 might have a role in male infertility. Further research in large cohorts with different populations is required to clarify the role of MDR in male fertility.

Key words: Male infertility; MDR1 (ABCB1); Genetic polymorphism; P-glycoprotein

INTRODUCTION

Infertility is a common problem occurring with increasing frequency, affecting 1 in 6 couples and approximately 1 in 25 men. Genetic factors are suggested to have important roles in male factor infertility because of the pattern of familial aggregation of this disorder. However, possible genetic etiologies are still not well understood.

The multidrug resistance protein 1 (*MRP1*) gene encodes a transporter protein called P-glycoprotein (P-gp), which is a member of a family of proteins of which only one subgroup has a role in multidrug resistance (MDR). *MDR1* is highly expressed in cancer cells and plays a key role in anticancer and antiviral therapy. It is also constitutively expressed in excretory and barrier tissues, including those of the intestines, liver, kidneys, pancreas, brain, testes, and placenta. It has protective and eliminatory roles and protects organisms against toxic substances, including xenobiotics, by eliminating such compounds from cells and preventing their accumulation (Mizuno et al., 2003; Leslie et al., 2005; Loscher and Potschka, 2005; Miller et al., 2008).

In the human testes, although not expressed in mature germ cells, P-gp is highly expressed at the luminal surface of capillary endothelial cells, as well as in Leydig cells, testicular macrophages, and Sertoli cells, which provide for the protection of somatic cells from potentially toxic substances. P-gp has been suggested to affect the microenvironment of the seminiferous tubules by transporting testicular steroids (Melaine et al., 2002). P-gp transports many xenobiotics including pesticides, which are well-known factors that decrease male fertility (Bain and LeBlanc, 1996; Tielemens et al., 1999; Marzolini et al., 2004).

The highly polymorphic *MDR1* gene is characterized by several single nucleotide polymorphisms (SNPs). The C3435T SNP in exon 26 of *MDR1* is a silent yet extensively studied polymorphism that is associated with low P-gp expression in enterocytes and peripheral blood mononuclear cells (PBMCs) (Owen et al., 2004). This SNP has an effect on both the expression and function of the P-gp protein (Hoffmeyer et al., 2000). In individuals homozygous for the T-allele, P-gp displays a low expression in intestinal and PBMCs, whereas homozygosity for the C-allele is associated with increased P-gp expression (Brinkmann et al., 2001).

The C3435T polymorphism (exon 26, C>T, Ile 1145 Ile) presents strong linkage disequilibrium with the C1236T synonymous SNP on exon 12 (C>T, Gly 412 Gly) (Kim et al.,

2001). These represent the most extensively analyzed synonymous *MDR1* SNPs for different diseases. Investigation of the genetic variations of the *MDR1* gene in diseases bears significance for the interpretation of the consequences such polymorphisms generate for P-gp function and disease etiology.

To our knowledge, although several studies exist on the association of diseases, including lung cancer, AML, colorectal cancer, esophageal cancer, inflammatory bowel disease (IBW), and Parkinson's disease (Tan et al., 2005; Annese et al., 2006; Gervasini et al., 2006; Kim et al., 2006; Komoto et al., 2006, Huebner et al., 2009; Balcerczak et al., 2010) with *MDR* polymorphisms, only one study is available on the association of the *MDR1* 3435 C>T polymorphism with male infertility, based on a Polish population (Drodźik et al., 2009). In our study we aimed to analyze the *MDR1* SNPs 3435 C>T and 1236 C>T in the Turkish male infertile population.

MATERIAL AND METHODS

We recruited 192 men with primary infertility and impaired seminal parameter(s) (mean age 32 years, range 25-41 years), known to have not achieved a pregnancy within at least one year of unprotected intercourse. A full clinical evaluation, including medical and reproductive history and physical examination of the genitals, was performed on the subjects. A minimum of two consecutive semen analyses were performed following a sexual abstinence of 3-5 days, in accordance with published criteria (World Health Organization, 2010). Each of the subjects presented with a history of infertility, lacking any indication of possible hormonal, infective, or physical causes. The only defect their clinical evaluation pointed out was the presence of one or more of the abnormal seminal parameters (density, motility, and/or morphology), referred to as male factor infertility. For the adjustment of the results, the subjects were questioned regarding their smoking habits, considering that it is the most common and influential health risk factor for this disorder. The subjects declaring to have more than one cigarette daily were considered smokers (Pasqualotto et al., 2006). Peripheral blood karyotype and Y chromosome analyses were performed by standard techniques in infertile subjects. The presence of varicoceles was clinically assessed based on genital examination by an infertility specialist (K.A.) and in suspected cases confirmed by a color Doppler ultrasound examination of the scrotum. Subjects detected to have varicocele, Y chromosome deletions, abnormal karyotypes, hypogonadotropic hypogonadism, or seminal tract obstructions were not included in the study. The history of their female partners was also recorded. General, systemic, and local clinical examinations were performed. The female partners of all the infertile men included in this study were investigated thoroughly and found to be healthy.

In the patient group of 100 infertile men whose sperm parameters were known, the genotyping results were found to be correlated with both the seminal parameters and the sperm DNA fragmentation results. The control group consisted of 102 healthy unrelated volunteers having at least one child without any assisted reproductive methods, revealing no medical history of cancer or other chronic diseases. The blood samples of the control group (mean age 36 years, range 27-47 years) were obtained from the Blood Banking Services of Ankara University. All the patients and controls were of Turkish ethnicity.

The study was approved by the Institutional Ethical Committee of Ankara University Faculty of Medicine (approval number 14-316), and written informed consent was obtained from all subjects who participated in the study.

Genotyping of *MDR1* polymorphisms

The genomic DNA samples used for polymorphic analyses were extracted from donor lymphocytes, using a standard method (Sambrook et al., 1982). *MDR1* genotyping was performed by polymerase chain reaction (PCR)-based restriction fragment length gene polymorphism. DNA amplification was performed under similar conditions for each polymorphism with the use of PCR buffer (MBI Fermentas, St Leon-Rot, Germany), 25 mM magnesium chloride (MBI Fermentas), 2.5 mM of each deoxyribonucleoside triphosphate (Sigma, St. Louis, MO, USA), 600 mg/mL DNA, 100 ng/mL of each specified primer (Iontek, Turkey), 0.5 mU Taq DNA polymerase (MBI Fermentas) in a total volume of 50 mL. After an initial denaturation at 94°C for 5 min, 35 cycles of 45 s at 94°C, 45 s at 57°-60°C, 1 min at 72°C, and a final extension period of 5 min at 72°C were carried out in a Corbett thermal cycler (Corbett Research, Sydney, Australia). Primer sequences, restriction enzymes, and fragment lengths are given in Table 1. DNA fragments generated were separated on a 3% NuSieve agarose gel. After the gel was stained with ethidium bromide (0.5 mg/mL), restriction fragments were visualized under a UV transilluminator.

Table 1. Positions of *MDR1* polymorphisms, primer sequences, restriction enzymes, and digestion conditions used in this study.

SNP	Primers	Restriction enzymes	Digestion conditions	PCR product and restriction fragment sizes
C1236T	F: 5'-TAT CCT GTG TCT GTG AAT TGC C-3' R: 5'-CCT GAC TCA CCA CAC CAA TG-3'	*Hae*III	37.8°C for 16 h	PCR: 366 bp, C allele: 269, 62, and 35 bp, T allele: 269 and 97 bp
C3435T	F: 5'-TGT TTT CAG CTG CTT GAT GG-3' R: 5'-AAG GCA TGT ATG TTG GCC TC-3'	*Sau*3AI	37.8°C for 16 h	PCR: 197 bp, C allele:158 and 39 bp, T allele: 197 bp

SNP = single nucleotide polymorphism; PCR = polymerase chain reaction.

Determination of sperm DNA damage using the comet assay

After undergoing a freeze-thaw cycle, semen samples were analyzed using the comet assay. The comet assay was performed on 102 semen samples obtained from male patients with infertility. Sperm DNA fragmentation was assessed using the single-cell gel electrophoresis (Comet) assay, as modified by Singh et al. (1988). Sperm cells stained with ethidium bromide were visualized under a fluorescence microscope (Olympus LX51, Olympus, Tokyo, Japan). Each cell with fragmented DNA had the appearance of a comet with a brightly fluorescent head and a tail to one side formed by the DNA, which contained strand breaks that were drawn away during electrophoresis. At least 300 sperm were counted for each sample. Each image was classified according to the intensity of the fluorescence in the comet tail and given a value of 0, 1, 2, 3, or 4 [from undamaged (class 0) to maximally damaged (class 4)], such that the total score of the slide would be between 0 and 400 arbitrary units (Gulum et al., 2011).

Statistical analyses

Continuous variables are reported as median (minimum-maximum) and means ± standard deviation. The Kruskal-Wallis test was used to compare the genotype groups for sperm DNA damage, classified as undamaged, low-damaged, moderately damaged, damaged,

or highly damaged. When the P value resulting from the Kruskal-Wallis test was significant, pairwise comparisons were performed. Allele and genotype frequencies of the patients with infertility and controls were compared with the chi-square test and the Fisher exact test, where applicable. Data analyses were performed using IBM SPSS Statistics v.20 (SPSS, Chicago, IL, USA). A P value < 0.05 was considered as statistically significant. Allele, genotype, and haplotype frequencies, consistency with Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium were analyzed with the SHEsis software (Shi and He, 2005).

RESULTS

In this study, we compared the infertile and the fertile control groups for the same two SNPs on the *MDR1* gene. The frequencies of the C and T alleles of the 1236 locus were found to be 61.2% (N = 235), 38.8% (N = 149); and 59.8% (N = 122), 40.2% (N = 82), in the infertile and the control groups, respectively. The C allele was detected more frequently in the patient group, although the difference was not significant (P > 0.05). The frequencies of the *MDR1* 1236 CC, CT, and TT genotypes were 34.4% (N = 66), 53.6% (N = 103), 12% (N = 23), in the infertile group; and 34.3% (N = 35), 51% (N = 52), 14.7% (N = 15) in the control group, respectively. However, the observed genotype frequencies did not show significant differences in either group (P > 0.05). The genotype frequency distribution of the 1236 locus was consistent with HWE in both groups (P > 0.05) (Table 2).

Table 2. Comparison of the genotype frequencies of the C3435T and C1236T loci between patients with infertility and controls.

SNP	Genotype	Patients N (freq)	Controls N (freq)	χ^2	Global P value	P value for patient HWE	P value for control HWE	Allele	Patients N (freq)	Controls N (freq)	χ^2	Global P value	Odds ratio (95%CI)
C1236T	CC	66 (0.34)	35 (0.34)	0.473	0.789	0.02*	0.845	C	235 (0.61)	122 (0.59)	0.108	0.741	1.060 (0.749-1.499)
	CT	103 (0.53)	52 (0.51)					T	149 (0.38)	82 (0.40)			
	TT	23 (0.12)	15 (0.14)										
C3435T	CC	53 (0.27)	27 (0.26)	4.077	0.130	0.07	0.542	C	216 (0.56)	104 (0.51)	1.491	0.221	1.23 (0.879-1.737)
	CT	110 (0.57)	50 (0.49)					T	168 (0.43)	100 (0.49)			
	TT	29 (0.15)	25 (0.24)										

HWE P values were calculated using the SHEsis program. SNP = single nucleotide polymorphism; Freq = frequency; HWE = Hardy-Weinberg equilibrium; CI = confidence interval. *Significant P value.

The C and T allele frequencies at the 3435 locus were found to be 56.2% (N = 216), 43.8% (N = 168); and 51% (N = 104), 49% (N = 100), in the infertile and the control groups, respectively. The differences between the infertile patient and the control groups were statistically insignificant (P > 0.05). The frequencies of the *MDR1* 3435 CC, CT, and TT genotypes were 27.6% (N = 53), 57.3% (N = 110), 15.1% (N = 29), in the infertile group; and 26.5% (N = 27), 49% (N = 50), 24.5% (N = 25), in the control group, respectively (Table 2). The observed genotype frequencies did not show any significant difference in either group (P > 0.05). The frequency of the 3435CT genotype was higher but statistically insignificant in the infertile group (P > 0.05). The genotype frequency distribution of the 3435 locus was consistent with HWE in the control group; however, it showed significant deviation from the HWE in the infertile group (P = 0.07).

The haplotype frequencies of the 1236 and 3435 loci are presented in Table 3. Each of the four possible haplotypes was noted in both the infertile and control groups. When the

frequency distributions of the estimated haplotypes were compared between the infertile and control groups, the frequency of the T-T haplotype was found to be significantly higher in the control group than in the infertile group [13.2 *vs* 6.5%, odds ratio (OR) = 0.459, P = 0.006].

Table 3. Haplotype analysis for the C1236T and C3435T polymorphic loci using the SHEsis software.

Haplotype	Patients [N (freq); total = 384]	Controls [N (freq); total = 204]	χ^2	Fisher's P	Pearson's P	Odds ratio (95%CI)
C-C	92 (0.240)	48 (0.240)	0.000	0.998	0.998	1.000 (0.672-1.488)
C-T	123 (0.323)	55 (0.270)	1.750	0.185	0.185	1.288 (0.885-1.876)
T-C	142 (0.372)	73 (0.358)	0.112	0.737	0.737	1.062 (0.746-1.512)
T-T	25 (0.065)	26 (0.132)	7.361	0.006*	0.006	0.459 (0.259-0.814)

d': 0.503, r^2: 0.137. CI = confidence interval. *Significant P value.

We investigated the correlation between the *MDR1* C3435T and C1236T polymorphisms and the risk of infertility. In the overall population, the correlation was analyzed in all genotype models (Table 4). We found that only the TT *vs* CT genotype comparison showed statistically significant differences in terms of association between the C3435T polymorphism and infertility (P = 0.045); there were no other significant differences between the groups. Our results indicated that the CT genotype might be a susceptibility factor for infertility and that the CT genotype was associated with high levels of sperm DNA damage (P = 0.02) (Table 5).

Table 4. Correlation of the *MDR1* C3435T and C1236T polymorphisms with the risk of infertility.

		OR	(95%CI)	P value
C3435T	CT *vs* CC	1.21	0.633-1.985	0.696
	TT *vs* CC	0.591	0.291-1.99	0.144
	TT *vs* CT	1.897	1.010-3.56	0.045*
	TT+CT *vs* CC	0.944	0.549-1.623	0.835
C1236T	CT *vs* CC	1.050	0.619-1.782	0.855
	TT *vs* CC	0.813	0.377-1.754	0.598
	TT *vs* CT	1.292	0.622-2.681	0.492
	TT+CT *vs* CC	0.997	0.601-1.654	0.992

OR = odds ratio; CI = confidence interval. *Significant P value.

Table 5. Correlation of the genotypes of the C3435T and C1236T loci with the total comet assay score.

SNP		CC	CT	TT	P value
C3435T	Mean ± SD	182.8 ± 53.8	215.2 ± 52.8*	166.5 ± 68.7	0.02*
	Med	180.7	206.7	153.5	
	(Min-Max)	(86.9 ± 305.5)	(133.6 ± 382.6)*	(62.8 ± 265.5)	
C1236T	Mean ± SD	193.3 ± 55.2	204.6 ± 59.3	195.0 ± 57.1	0.587
	Med	201.5	205.2	192.7	
	(Min-Max)	(76.2 ± 329.2)	(62.8 ± 382.6)	(142.0 ± 256.3)	

SNP = single nucleotide polymorphism; SD = standard deviation; med = median; min = minimum; max = maximum. *Significant P value.

In this survey, binary genotypic analysis was carried out for the nine potential combinations of the two loci (Table 6). In both the infertile patient and control groups, CC-CT, CT-CC, and CT-CT were detected as the three most common genotype combinations with frequencies of 19.3, 19.8, and 31.8% in the infertile group, and 16.7, 16.7, and 27.5% in the control group, respectively. Genotype combinations from the two SNPs did not significantly differ between the infertile and fertile groups (P = 0.357).

Table 6. Comparative analysis of binary genotypes observed for the *MDR1* C3435T-C1236T loci in the patients with infertility and controls.

Genotypes	Patients		Controls		P value	OR (95%CI)
	N	Frequency	N	Frequency		
CC-CC	5	0.0260	4	0.0392	0.724	1.524 (0.401-5.814)
CC-CT	37	0.1927	17	0.1666	0.583	0.838 (0.445-1.577)
CC-TT	11	0.0572	6	0.0588	0.957	1.028 (0.369-2.866)
CT-CC	38	0.1979	17	0.1666	0.513	0.811 (0.431-1.522)
CT-CT	61	0.3177	28	0.2745	0.443	0.813 (0.487-1.381)
CT-TT	11	0.0572	5	0.0490	0.766	0.848 (0.286-0.251)
TT-CC	23	0.1197	14	0.1372	0.667	1.169 (0.573-2.386)
TT-CT	5	0.0260	7	0.0686	0.118	2.754 (0.851-8.928)
TT-TT	1	0.0052	14	0.0392	0.051	7.812 (0.859-7.1428)

OR = odds ratio; CI = confidence interval.

We investigated 100 infertile men whose semen parameters were known to analyze the effects of the C3435T and C1236T polymorphisms on sperm parameters (morphology, motility, and concentration) and DNA fragmentation. No correlation was found to exist between polymorphisms of the *MDR1* gene and sperm morphology, motility, or concentration (Table 7). However, the 3435 CT genotype was determined to be correlated with increased DNA damage levels of sperm ($P < 0.05$) (Table 5).

Table 7. Correlation of the genotypes of the C3435T and C1236T loci with sperm parameters.

	C3435T			C1236T		
	CC	CT	TT	CC	CT	TT
Sperm count						
≥ 15 million/mL	13 (44.8%)	23 (38.3%)	5 (45.5%)	16 (47.1%)	23 (37.1%)	2 (50.0%)
< 15 million/mL	16 (55.2%)	37 (61.7%)	6 (54.5%)	18 (52.9%)	39 (62.9%)	2 (50.0%)
		P value 0.802			P value 0.594	
Progressive Motility						
≥ 32 %	16 (55.2%)	32 (53.3%)	8 (72.7%)	20 (58.8%)	34 (54.8%)	2 (50.0%)
< 32 %	13 (44.8%)	28 (46.7%)	3 (27.3%)	14 (41.2%)	28 (45.2%)	2 (50.0%)
		P value 0.489			P value 0.904	
Kruger						
≥ 4 %	3 (10.3%)	13 (21.7%)	5 (45.5%)	9 (26.5%)	11 (17.7%)	1 (25.0%)
< 4 %	26 (89.7%)	47 (78.3%)	6 (54.5%)	25 (73.5%)	51 (82.3%)	3 (75.0%)
		P value 0.051			P value 0.592	

DISCUSSION

Several studies have reported that both the quality and quantity of human semen have significantly decreased over the past decades (Geoffroy-Siraudin et al., 2012). The underlying reasons remain unknown. Many environmental, physiologic, and genetic factors may impair reproductive functions in men. Among the environmental factors, xenobiotics have been shown to play a significant role. Biologically active xenobiotics produce adverse effects by means of covalent interactions between intermediate metabolites and cellular macromolecules, including DNA and protein. Xenobiotics act as substrates for P-gp, which is produced by the *MDR1* (*ABCB1*) gene. P-gp is found in the blood-testis barrier and protects the testes by preventing the penetration of xenobiotics. *Mdr1a (–/–)* knockout mice have been shown to accumulate more P-gp substrates (e.g., ivermectin, vinblastine, and nelfinavir) within the testicular tissue than do wild-type controls (Schinkel, 1997; Choo et al., 2000).

Genetic polymorphisms of the *MDR1* gene were first identified by Kioka et al. (1989) Alterations in P-gp expression and function potentially depend on variations of the *MDR1* nucleotide sequence. The most commonly reported *MDR1* SNPs in the P-gp are the synonymous SNPs 1236 (exon 12, C>T, Gly 412 Gly) and 3435 (exon 26, C>T, Ile 1145 Ile). Although the C3435T mutation is a silent mutation, this polymorphism affects the expression and function of the P-gp. The C3435T polymorphism presents strong linkage disequilibrium with the C1236T synonymous SNP on exon 12 (Kim et al., 2001). *MDR1* expression is highly variable between subjects. This variability demonstrates that the interethnic diversity and genetic polymorphism of the *MDR1* gene is associated with a variation in expression level (Hoffmeyer et al., 2000; Taniguchi et al., 2003; Meissner et al., 2004).

Only one study has been published on the association of the *MDR1* 3435 C>T polymorphism with male infertility, which was carried out in the Polish population by Droździk et al. (2009). In this study, only the relation between the *MDR1* 3435 C>T polymorphism and male infertility was analyzed. In the present study, we investigated the correlation between male infertility and both *MDR1* gene C1236T and C3435T polymorphisms, along with their haplotypes and binary genotypes. Both studies are based on the assumption that *MDR1* SNPs are associated with lower P-gp expression in the testes, leading to damage of the testes and eventually infertility. Droździk et al. (2009) reported that the risk of infertility was significantly elevated (two-fold) in individuals carrying at least one T allele, including individuals of the 3435TT and 3435CT genotypes. As the *MDR1* T allele was previously associated with lower P-gp activity, these results were interpreted as an indication of this allele possibly being related to the increased testicular penetration of xenobiotics. These results suggested that individuals carrying a T allele might be more prone to developing infertility. However, our results showed that the frequencies of the *MDR1* 3435 CT and TT genotypes among infertile and fertile subjects were 72.4 and 73.5%, respectively. Thus, we found no differences between the fertile and infertile groups in contrast to those seen in the Polish population. The difference between the results of the two studies might be due to interethnic diversity as well as due to the possibility that the observed association of the *MDR1* 3435T allele with infertility in the study of Droździk et al. (2009) might represent a false positive, as they indicated in the article. Furthermore, the frequency of *MDR1* 3435C>T genotypes among the control group of fertile men in the Droździk et al. study differed from published data for healthy controls from the same region (Kurzawski et al., 2006). Therefore, the significance of their results might arise from an overrepresentation of 3435CC homozygotes among the controls, as they suggested (Droździk et al., 2009).

We found that only the TT *vs* CT genotype comparison showed statistically significant differences ($P = 0.045$) in terms of association between the C3435T polymorphism and infertility (Table 4), and that there were no other significant differences between the groups. We also showed that the CT genotype was associated with high levels of sperm DNA damage. This result also supported the association between the CT genotype and infertility risk. In a study carried out by Rüstemoğlu et al. (2011) the CT genotype was also observed more frequently in the patient familial mediterranean fever group. It was suggested that the CT genotype might be a susceptibility factor for the disease phenotype. According to our result, we also suggested that the CT genotype might be a susceptibility factor for infertility. Bektaş-Kayhan et al. (2012) also described that children with acute lymphoblastic leukemia carrying the CT genotype are more prone to develop oral mucositis and might therefore also be more susceptible to the side effects of chemotherapy.

In the Polish population, no correlation was found between the *MDR1* C3435T poly-

morphism and sperm parameters. Similarly, we did not find any correlation between the *MDR1* SNPs 3435 or 1236 and sperm parameters. However, the CT genotype at the C3435T locus was more common in infertile men with high levels of sperm DNA damage (P = 0.02).

The frequencies of the *MDR1* 1236 and 3435 alleles and genotypes determined in our control group were in agreement with those reported in previous studies conducted in the Turkish population (Gümüş-Akay et al., 2010, Dogu et al., 2012; Rüstemoğlu et al., 2012).

Recent studies have demonstrated that inconsistencies might be better understood by grouping SNPs into haplotypes and this method might be used to predict the functional consequences of *MDR1* polymorphisms (Kimchi-Sarfaty et al., 2007). Our results showed that the frequency of the T-T haplotype was significantly higher in the control group (13.2 *vs* 6.5%; OR = 0.459, 95% confidence interval = 0.259-0.814, P = 0.006). Our data could be interpreted to mean that the T-T haplotype might serve as a protective factor for the infertility phenotype, as the frequency of this haplotype was found to be significantly lower in infertile men than in fertile controls (Table 3).

Our findings could indicate a new predictor of fertility. Further studies are required in a large series of different populations to clarify the role of MDR variation on male fertility.

Conflicts of interest

The authors declare no conflict of interest.

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