Genetic polymorphisms and haplotypes of the *DJ-1* gene promoter associated with the susceptibility to male infertility

Danial Jahantigh¹ · Abasalt Hosseinzadeh Colagar¹ · Saeedeh Salimi^{2,3}

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Abstract

Purpose In this study, we evaluate the relationship between genetic polymorphisms of the DJ-1 gene, g.-6_+10del, and g.168_185del with male infertility susceptibility.

Methods Four hundred and twenty-two male infertile patients and 285 fertile male controls were recruited. Genotyping was performed by polymerase chain reaction. In silico analysis was performed by EPD, ElemeNT, SNPnexus, and PROMO to predict the potential functions of rs901561484 and rs373653682 polymorphisms.

Results The Del (D) allele carriers of DJ-1 g.-6_+10del polymorphism were significantly associated with the risk of male infertility in total infertile, asthenozoospermia, and oligoasthenozoospermia patients. Moreover, the Del (D) allele of DJ-1 g.-6_+10del polymorphism significantly increased in total male infertile, asthenozoospermia, and oligoasthenozoospermia groups. In addition, the frequencies of different genotypes and the Del allele and Dup allele carriers of DJ-1 g.168_185del gene polymorphisms were associated with male infertility in total infertile and four different sub-group patients. Furthermore, haplotype analysis of DJ-1 g.-6_+10del and g.168_185del polymorphisms revealed that the D-Dup and I-Del haplotype frequencies significantly increased the risk of male infertility,

while I-Ins haplotypes were associated with a decreased risk of male infertility in total and sub-group patients. The in silico analysis showed that the presence of Ins and/or Dup alleles of the DJ-1 g.-6_+10del and g.168_185del polymorphisms could provide additional binding sites of more nuclear factors and probably affect transcriptional activity.

Conclusion(s) Our study presents evidence of a strong association between functional polymorphisms of the DJ-1 promoter, g.-6_+10del, and g.168_185del with the risk of male infertility.

Keywords DJ-1 · Male infertility · Ins/Del · Genetic polymorphism · In silico

Introduction

Male infertility is found in half of involuntarily childless couples, which affects approximately 7% of the male population worldwide [1]. The main causes of male infertility are deficits in the semen quality and quantity parameters including morphology, movement, and number of sperm [2]. The exact etiology of approximately half of all male infertility cases remains unknown, which is known as idiopathic infertility.

Strong evidences suggest that oxidative stress including reactive oxygen species (ROS) plays an important role in the pathogenicity and development of male infertility [3, 4]. ROS is naturally occurring and is produced in different cells during normal metabolism of oxygen and, also, via environmental stimulators [5]. In the male reproductive tract, surplus ROS concentrations, generated from the mitochondrial respiratory chain in spermatozoa and from leukocytes and macrophages, together with the malfunctioning of antioxidant systems can negatively affect concentration, motility, and morphology of

✉ Abasalt Hosseinzadeh Colagar
acolagar@yahoo.com; ahcolagar@umz.ac.ir

¹ Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar 47416-95447, Iran

² Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

³ Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

Fig. 1 *DJ-1* gene map and schematic and products of the *DJ-1* g.-6 + 10del (rs901561484) and g.168_185del (rs373653682) polymorphisms. Human *DJ-1* gene map was deduced from NCBI data bank with about 24 kb, eight exons, which rs901561484 and rs373653682 are shown in

promoter region (A). Schematic diagram of the *DJ-1* gene showing the position of the rs901561484 (B) and rs373653682 (C) polymorphisms along with various alleles. PCR amplified products of the rs901561484 (B') and rs373653682 (C') polymorphisms

sperm causing poor semen parameters and ultimately infertility [6–8]. Antioxidant defense systems are largely abundant in semen plasma or in sperm cells and are mandatory for normal spermatogenesis and fertilization [9, 10]. Furthermore, genetic variations in antioxidant genes may cause male infertility via influencing the level of gene expression and/or changes in folding leading eventually to dysfunction. Functional polymorphisms of antioxidant genes NRF2, SOD, GST, NOS, CAT, and GPX have been reported as being associated with male infertility risk [11].

DJ-1 is an approximately 20-kDa homodimer protein composed of two subunits of 189 amino acid residues ubiquitously expressed in various human cells and tissues, with a high expression in the testes and sperm [12]. DJ-1 is known as a multifunctional protein which plays a critical role in antioxidative stress reaction [13, 14], transcriptional regulation [15, 16], and mitochondrial function [17, 18]. DJ-1 directly and indirectly acts as a key regulator in the antioxidative defense system [19]. DJ-1 is coactivator or corepressor of several transcription factors such as nuclear factor erythroid-2-related factor 2 (Nrf2), which is a master transcription factor for oxidative stress and detoxication responses [20]. In addition, DJ-1 stimulates the expression of superoxide dismutase (SOD 3) and glutathione synthesis genes to reduce ROS levels [21, 22]. It has also been reported that DJ-1 directly binds to p53 to inhibit p53 transcriptional activity, leading to cell cycle

progression [15, 23]. After oxidative stress, DJ-1 inhibits oxidative stress-induced apoptosis and prevents cell death [24, 25], as well as, in an indirect way, activates the PI3 K/Akt pathway, thereby leading to cell growth [26, 27].

Furthermore, it has been demonstrated that DJ-1 is related to male infertility in rats and mice. The rat homolog of human DJ-1, CAP1 (contraception-associated protein 1), or SP22 (sperm protein 22) was exposed to sperm toxicants in which DJ-1/CAP1/SP22 levels in the sperm and epididymis decreased in parallel with the subsequent rat infertility [28–30]. Notably, it has been shown that DJ-1 that participates in fertilization allows sperm to penetrate into the zona pellucida of eggs and anti-SP22 or anti-DJ-1 antibodies are capable of significantly inhibiting in vitro fertilization [31–34]. The *DJ-1* gene is located on chromosome 1p36.2-p36.3; it spans about 24 kb and contains eight exons [35] (Fig. 1(A)). Mutations in the coding regions of this gene are rare, but two common indel polymorphisms in the promoter region of the *DJ-1* gene g.-6 + 10del (rs901561484) and g.168_185del (rs373653682) have been investigated as candidates in different genetic association studies [36–39].

Although the limited studies were focused on the expression level of DJ-1 in human spermatozoa [40, 41], the association between genetic polymorphism in DJ-1 with male infertility has not been reported to date. In the current study, we aimed to evaluate the possible relation between two indel

Table 1 The primer sequences and PCR conditions

Polymorphism name	5' to 3' oligonucleotide	Annealing temp., °C	PCR product
g.-6_+10del (rs901561484)	F: GAGGTAGACTCGGCCGGAC R: CAACGCTGAAGCGT CCAGAA	63.6	244 bp (Del); 260 bp (Ins)
g.168_185del (rs373653682)	F: GTTCATTTTCAGCCTGGTGTG R: CAACGCTGAAGCGT CCAGAA	59.3	288 bp (Del); 300 bp (Ins); 318 bp (Dup)

polymorphisms of *DJ-1* gene, g.-6_+10del (rs901561484), and g.168_185del (rs373653682), with male infertility risk in a case-control study among Iranian population parallel with an in silico analysis.

Material and methods

Patients and control samples

In this case-control study, a total of 422 idiopathic infertile patients, including 128 asthenozoospermia (< 25% motility or < 50% progression in a semen sample, and fresh sperm concentration > 20×10^6 /ml); 83 oligoasthenozoospermia (motility < 25%, and fresh sperm concentration < 20×10^6 /ml); 95 oligozoospermia (motility > 25%, and fresh sperm concentration < 20×10^6 /ml); 116 non-obstructive azoospermia (NOA) (without spermatozoa in the ejaculate semen); and 285 age-matched fertile men, were recruited from the infertility clinics of Fatemeh Zahra Hospital in Babol, Iran, between 2013 and 2015. The mean age of the infertile men was 34.26 ± 4.13 years and of the fertile subjects was 33.24 ± 5.71 . The subjects with known causes of infertility including history of cryptorchidism, orchitis, obstruction of the vas deferens, varicocele, infectious diseases, drug abuse, diabetes mellitus, abnormal hormone profile (LH, FSH, and testosterone), Y-chromosome microdeletion, and abnormal karyotype were excluded from the study. In addition, the subjects with a history of any genetic and familial disorders were excluded. The control group was composed of 285 fertile men with normal sperm parameters, without a history of infertility and at least one offspring. All patients underwent at least two semen analyses according to World Health Organization (WHO) guidelines after 3–5 days of sexual abstinence. The study was approved by the ethics committees of the University of Mazandaran and informed consent was obtained from each subject before participation.

DNA extraction and genotyping

Genomic DNA was extracted from blood samples, which were collected in 2-ml EDTA_{Na2} tubes from patients and healthy controls, by using the commercial available kit

(Cinnagen, Iran). The extracted DNA was stored at -20°C . The DJ-1 g.-6_+10del (rs901561484) and g.168_185del (rs373653682) gene polymorphisms were genotyped using polymerase chain reaction (PCR). To amplify the DJ-1 g.-6_+10del and g.168_185del fragments, PCR was carried out in 25 μl of PCR reaction containing 0.2 U, *Taq* polymerase; 0.2 μM , Mix dNTPs; 0.35 μM each of forward and reverse primer (Table 1); 1.5 μM , MgCl₂; and 30 ng of template DNA. All PCR reagents were purchased from SinaClon Co, Iran.

The PCR products for DJ-1 g.-6_+10del polymorphism were 244 and 260 bp which refer to Del (D) allele and Ins (I) allele, respectively. The fragment size for DJ-1 g.168_185del polymorphism was 288, 300, and 318 bp which were for Del allele, Ins allele, or Dup allele, respectively. PCR products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide staining. More than 30% of the samples were chosen at random for repeated assays, and the results were 100% consistent.

In silico analysis

In silico analyses were performed to evaluate potential biological functions of two promoter indel polymorphisms, rs901561484, and rs373653682, located in the promoter region of DJ-1 gene. For detection of core promoter motifs including TATA box, Initiator, GC box, and CCAAT box, the promoter sequences of that DJ-1 gene contain g.-6_+10del and g.168_185del indel polymorphisms were screened by the prediction tools EPD [42] and Element [43]. Besides, SNPnexus and PROMO were used to find potential transcription factor binding sites in SNPs containing promoter sequences, as previously described [44].

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was calculated for both the infertile and control groups. All data were analyzed using SPSS software version 18. Differences in the frequency of alleles and genotypes were analyzed using the chi-square test or Fisher's exact test. The association between DJ-1 gene polymorphisms and male infertility was estimated by computing the odds ratio (OR) and 95% confidence intervals (CI)

Table 2 Genotype and allele frequencies of DJ-1 promoter in controls and total infertile patients

Genotype	No. and percentage		<i>p</i> value [OR (95% CI)]
	Fertile (<i>n</i> = 285)	Infertile (<i>n</i> = 422)	
g.-6_+10del			
I/I	264 (92.63)	369 (87.44)	
I/D	17 (5.96)	38 (9.00)	0.120 [1.599 (0.883–2.894)]
D/D	4 (1.75)	15 (3.55)	0.082 [2.682 (0.880–8.175)]
D carriers	21 (7.36)	53 (12.55)	0.028 [1.805 (1.063–3.066)]
I	545 (95.61)	776 (91.94)	
D	25 (4.38)	68 (8.05)	0.007 [1.910 (1.192–3.060)]
g.168_185del			
Ins/Ins	169 (59.29)	171 (40.52)	
Ins/Del	44 (15.43)	92 (21.80)	0.0007 [2.066 (1.361–3.137)]
Del/Del	6 (2.10)	31 (7.34)	0.0004 [5.106 (2.076–12.55)]
Ins /Dup	25 (8.77)	59 (13.98)	0.0012 [2.332 (1.395–3.898)]
Del/Dup	2 (0.70)	6 (1.42)	0.187 [2.964 (0.590–14.89)]
Dup/Dup	39 (13.68)	63 (14.92)	0.042 [1.596 (1.015–2.509)]
Del carriers	52 (18.24)	129 (30.56)	< 0.0001 [2.451 (1.666–3.606)]
Dup carriers	66 (23.15)	128 (30.33)	0.0005 [1.916 (1.330–2.761)]
Ins	407 (71.40)	493 (58.41)	
Del	58 (10.17)	160 (18.95)	< 0.0001 [2.277 (1.640–3.160)]
Dup	105 (18.42)	191 (22.63)	0.0034 [1.501 (1.144–1.971)]

Significant differences between the case and control groups are italicized

OR odds ratio, CI confidence interval

from a logistic regression analysis model after adjustment for age. The haplotypes were assigned using the online software platform SHEsis (<http://www.analysis.bio-x.cn>). The haplotype construction element was based on the standard Full-Precise-Iteration (FPI) algorithm [45]. All tests were two-tailed, and *p* values < 0.05 were considered as statistically significant.

Results

Genotype analysis of *DJ-1* promoter indel gene polymorphisms in different male infertile groups

The *DJ-1* g.-6_+10del polymorphism contains two different alleles including Del (D) allele and Ins (I) allele, 244 and 260 bp, respectively, (Fig. 1(C and C')). The distributions of allelic and genotypic frequencies for *DJ-1* g.-6_+10del polymorphism have been summarized in Tables 2 and 3. Statistical analysis showed significant differences between total infertile, asthenozoospermia, oligoasthenozoospermia, oligozoospermia, and non-obstructive azoospermia groups compared with control groups.

The frequencies of I/D and D/D genotypes of *DJ-1* g.-6_+10del polymorphism were approximately 1.5- and 2.5-fold higher in the total infertile group compared with the controls,

but those differences were not statistically significant (*p* = 0.120 and *p* = 0.082, respectively). A significant difference was observed between the Del (D) allele carriers in total infertile men compared to fertile controls, so that the risk of infertility was 1.8-fold higher in individuals with the Del (D) allele (*p* = 0.028) (Table 2). Moreover, the Del (D) allele frequency of *DJ-1* g.-6_+10del showed approximately 2-fold increased risk of total infertility and was statistically different (*p* = 0.007).

After sorting the infertile patients according to the deficiencies in movement and/or amount of sperm in four separate groups, the asthenozoospermia, oligoasthenozoospermia, oligozoospermia, and non-obstructive azoospermia, the frequency of D/D genotype of *DJ-1* g.-6_+10del polymorphism increases approximately 5-fold the risk of asthenozoospermia compared to fertile controls (*p* = 0.010). Moreover, highly significant differences in frequencies of the Del (D) allele carriers were found between asthenozoospermia and oligoasthenozoospermia groups compared with the controls (*p* = 0.003 and *p* = 0.001, respectively). The frequency of the Del (D) allele of *DJ-1* g.-6_+10del polymorphism observed was significantly higher than the Ins (I) allele in asthenozoospermia and oligoasthenozoospermia patients compared to the control group (*p* = 0.0002 and *p* = 0.0025, respectively).

As it has been shown in Fig. 1(B and B'), the *DJ-1* g.168_185del polymorphism contains three different alleles

Table 3 Genotype and allele frequencies of DJ-1 promoter in controls and four different sub-group infertile patients

Genotype	Control (<i>n</i> = 285) <i>n</i> (%)	Patients				<i>p</i> value [OR (95% CI)]	Controls vs. AS	Controls vs. OAS	Controls vs. Oligo	Controls vs. NOA
		AS (<i>n</i> = 128) <i>n</i> (%)	OAS (<i>n</i> = 83) <i>n</i> (%)	Oligo (<i>n</i> = 95) <i>n</i> (%)	NOA (<i>n</i> = 116) <i>n</i> (%)					
g.-6_+10del										
I/I	264 (92.63)	106 (82.81)	69 (83.13)	89 (93.68)	105 (90.51)					
I/D	17 (5.96)	14 (10.93)	10 (12.04)	6 (6.31)	8 (6.89)	0.057 [2.051 (0.976–4.309)]	0.053 [2.250 (0.986–5.134)]	0.925 [1.046 (0.400–2.737)]		0.704 [1.183 (0.495–2.824)]
D/D	4 (1.75)	8 (6.25)	4 (4.81)	–	3 (2.58)	0.010 [4.981 (1.468–16.89)]		0.456 [0.328 (0.017–6.159)]		0.411 [1.885 (0.414–8.570)]
D carriers	21 (7.36)	22 (17.18)	17 (20.48)	6 (6.31)	11 (9.48)	0.003 [2.609 (1.377–4.943)]	0.001 [3.097 (1.550–6.188)]	0.729 [0.847 (0.331–2.166)]		0.479 [1.317 (0.613–2.826)]
I	545 (95.61)	226 (88.28)	148 (89.15)	184 (96.84)	218 (93.96)					
D	25 (4.38)	30 (11.71)	18 (10.84)	6 (3.15)	14 (6.03)	0.0002 [2.893 (1.664–5.030)]	0.0025 [2.651 (1.408–4.990)]	0.460 [0.710 (0.287–1.760)]		0.327 [1.400 (0.714–2.743)]
g.168_185del										
Ins/Ins	169 (59.29)	62 (48.43)	30 (41.86)	41 (46.15)	38 (32.75)					
Ins/Del	44 (15.43)	25 (19.53)	19 (20.93)	24 (24.61)	24 (20.68)	0.133 [1.548 (0.875–2.740)]	0.008 [2.432 (1.252–4.723)]	0.008 [2.248 (1.230–4.109)]		0.004 [2.425 (1.319–4.461)]
Del/Del	6 (2.10)	9 (7.03)	8 (9.30)	4 (3.07)	10 (8.62)	0.010 [4.088 (1.398–11.95)]	0.0005 [7.511 (2.432–23.19)]	0.130 [2.748 (0.741–10.18)]		0.0002 [7.412 (2.538–21.64)]
Ins/Dup	25 (8.77)	17 (13.28)	10 (11.62)	14 (15.38)	18 (15.51)	0.075 [1.853 (0.937–3.663)]	0.055 [2.253 (0.982–5.166)]	0.026 [2.308 (1.103–4.827)]		0.001 [3.202 (1.589–6.452)]
Del/Dup	2 (0.70)	1 (0.78)	4 (6.97)	–	1 (0.086)	0.801 [1.362 (0.121–15.29)]	0.006 [11.26 (1.975–64.27)]	0.896 [0.816 (0.038–17.34)]		0.518 [2.223 (0.196–25.16)]
Dup/Dup	39 (13.68)	14 (10.93)	12 (9.30)	12 (10.76)	25 (21.55)	0.949 [0.978 (0.497–1.924)]	0.153 [1.733 (0.815–3.686)]	0.524 [1.268 (0.610–2.635)]		0.0008 [2.850 (1.544–5.263)]
Del carriers	52 (18.24)	35 (27.34)	31 (37.20)	28 (27.69)	35 (30.17)	0.021 [1.834 (1.092–3.079)]	0.0001 [3.358 (1.860–6.060)]	0.006 [2.219 (1.252–3.933)]		0.0001 [2.993 (1.719–5.211)]
Dup carriers	66 (23.15)	32 (25.00)	26 (25.58)	26 (26.15)	44 (37.93)	0.286 [1.321 (0.791–2.207)]	0.008 [2.219 (1.221–4.032)]	0.094 [1.623 (0.920–2.865)]		< 0.0001 [2.964 (1.764–4.982)]
Ins	407 (71.40)	166 (64.28)	89 (58.13)	120 (66.15)	118 (50.86)					
Del	58 (10.17)	44 (17.34)	39 (23.25)	32 (15.38)	45 (19.39)	0.004 [1.860 (1.208–2.863)]	< 0.0001 [3.075 (1.929–4.901)]	0.010 [1.871 (1.161–3.015)]		< 0.0001 [2.676 (1.723–4.155)]
Dup	105 (18.42)	46 (18.36)	38 (18.60)	38 (18.46)	69 (29.74)	0.719 [1.074 (0.726–1.587)]	0.023 [1.655 (1.069–2.560)]	0.342 [1.227 (0.803–1.874)]		< 0.0001 [2.266 (1.571–3.269)]

Significant differences between the case and control groups are italicized

OR odds ratio, CI confidence interval, AS asthenozoospermia, OAS oligoasthenozoospermia, Oligo oligozoospermia, NOA non-obstructive azoospermia

Table 4 Haplotypes of the DJ-1 promoter in controls and total infertile patients

rs901561484	rs373653682	Fertile (%)	Infertile (%)	<i>p</i> value [OR (95% CI)]
D	Dup	25 (0.044)	68 (0.081)	<i>0.0063</i> [<i>1.910 (1.192–3.061)</i>]
I	Ins	407 (0.714)	493 (0.584)	<i>< 0.0001</i> [<i>0.563 (0.448–0.706)</i>]
I	Del	58 (0.102)	160 (0.190)	<i>< 0.0001</i> [<i>2.065 (1.497–2.848)</i>]
I	Dup	80 (0.140)	123 (0.146)	0.7770 [1.045 (0.771–1.416)]

Significant differences between the case and control groups are italicized

OR odds ratio, CI confidence interval

including Del allele, Ins allele, and Dup allele, 282, 300, and 318 bp, respectively. The allele and genotype frequencies of DJ-1 g.168_185del polymorphism in male infertile patients and controls are summarized in Tables 2 and 3. With regard to DJ-1 g.168_185del, the Ins/Del, Del/Del, Ins/Dup, and Dup/Dup genotypes in total infertile men were significantly higher than fertile controls ($p = 0.0007$, $p = 0.0004$, $p = 0.0012$, and $p = 0.042$, respectively). Additionally, the frequencies of the Del allele and Dup allele carriers were approximately 2.5- and 2-fold higher in total infertile compared to the control group, which were highly significant ($p < 0.0001$ and $p = 0.0005$, respectively). Also, the Del allele and Dup allele frequencies in total infertile patients were statically different than the controls ($p < 0.0001$ and $p = 0.0034$, respectively).

After dividing the infertile patients to four different sub-groups, the Ins/Del genotype frequencies showed an increase of more than 2-fold in oligoasthenozoospermia, oligozoospermia, and non-obstructive azoospermia compared to fertile men; these increases were highly significant ($p = 0.008$, $p = 0.0008$, and $p = 0.004$, respectively). The frequencies of the Del/Del genotypes showed an increase of a 4-fold risk of asthenozoospermia and, also, an increase of approximately 7.5-fold of oligoasthenozoospermia and non-obstructive azoospermia compared with the control group; again, these increases were statistically significant ($p = 0.010$, $p = 0.0005$, and $p = 0.0002$, respectively). The frequency of the Del/Dup genotype was significantly higher in oligoasthenozoospermia patients (6.97%) compared to fertile controls (0.70%) and risk of male infertility was more than 11-fold higher in this group ($p = 0.006$) (Table 3). Moreover, a highly significant difference was observed in the Dup/Dup genotype frequency between non-obstructive azoospermia patients and the controls ($p = 0.0008$).

The frequencies of individuals with Del allele (Ins/Del, Del/Del, and Del/Dup genotypes) significantly increased risk of male infertility in each of the four different sub-groups ($p = 0.021$, $p = 0.0001$, $p = 0.006$, and $p = 0.0001$, respectively) (Table 3). Likewise, the Dup allele carriers showed a high significant increase in oligoasthenozoospermia and non-obstructive azoospermia patients compared to fertile men ($p = 0.008$ and $p < 0.0001$, respectively).

Notably, the frequencies of Del allele were significantly increased in each of the four different sub-groups compared

to the control group ($p = 0.004$, $p < 0.0001$, $p = 0.010$, and $p < 0.0001$, respectively); also, the allele Dup frequencies have shown significant differences in oligoasthenozoospermia and non-obstructive azoospermia patients compared to fertile men ($p = 0.023$ and $p < 0.0001$, respectively).

Haplotype analysis of DJ-1 promoter indel gene polymorphisms

Haplotypes were constructed in male infertility and controls and the haplotypes with the frequency of $> 3\%$ were built from DJ-1 rs901561484 and rs373653682. Four homozygous haplotypes of the DJ-1 promoter, namely D-Dup, I-Ins, I-Del, and I-Dup, were identified in all subjects (Tables 4 and 5). The D-Dup and I-Del haplotype frequencies shown an increase of approximately 2-fold in the total infertile patients compared fertile men, and these increases were statically significant ($p = 0.0063$ and $p < 0.0001$, respectively). Besides, the I-Ins haplotype frequency was significantly lower in the total infertile patients and showed a protective role ($p < 0.0001$).

Additional haplotype analysis was performed for four different sub-groups as summarized in Table 5. Similar to the total infertile results, the D-Dup and I-Del haplotype frequencies significantly increased the risk of male infertility so that the D-Dup haplotypes showed an increase of approximately 2- and 3-fold in oligoasthenozoospermia and asthenozoospermia, $p = 0.0405$ and $p < 0.0001$, respectively. Also, the I-Del haplotypes shown an increase of 1.8-, 2.6-, 1.7-, and 2.1-fold in the four different male infertile sub-groups compared to fertile controls, $p = 0.0046$, $p < 0.0001$, $p = 0.0137$, and $p = 0.0004$, respectively.

After dividing the infertile patients to four different sub-groups, the I-Ins haplotypes retained their protective roles in oligoasthenozoospermia, oligozoospermia, and non-obstructive azoospermia, $p < 0.0001$, $p = 0.0327$, and $p < 0.0001$, respectively, as total infertile patient results (Tables 4 and 5). Although the I-Dup haplotype frequency did not show statistical difference between total infertile patients compared with controls (Table 4), it revealed conflicting results in other sub-groups. No differences were detected in the I-Dup haplotype frequencies between oligoasthenozoospermia and oligozoospermia with controls, and this haplotype showed a protective role in

Table 5 Haplotypes of the DJ-1 promoter in controls and four different sub-group infertile patients

Haplotypes	Control (%)		Patients		<i>p</i> value [OR (95% CI)]							
	AS (%)	OAS (%)	Oligo (%)	NOA (%)	Controls vs. AS	Controls vs. OAS	Controls vs. Oligo	Controls vs. NOA				
D-Dup	25 (0.044)	30 (0.117)	14 (0.084)	6 (0.032)	14 (0.60)	< 0.0001 [2.894 (1.665–5.030)]	0.0405 [2.008 (1.019–3.957)]	0.4586 [0.711 (0.287–1.760)]	0.3250 [1.400 (0.714–2.744)]			
I-Ins	407 (0.714)	166 (0.648)	90 (0.542)	120 (0.632)	118 (0.509)	0.0585 [0.739 (0.539–1.012)]	< 0.0001 [0.474 (0.332–0.667)]	0.0327 [0.687 (0.486–0.971)]	< 0.0001 [0.415 (0.303–0.568)]			
I-Del	58 (0.102)	44 (0.172)	38 (0.229)	32 (0.168)	45 (0.194)	0.0046 [1.832 (1.200–2.798)]	< 0.0001 [2.621 (1.667–4.121)]	0.0137 [1.788 (1.121–2.852)]	0.0004 [2.124 (1.390–3.246)]			
I-Dup	80 (0.140)	15 (0.063)	24 (0.145)	32 (0.168)	55 (0.237)	0.0012 [0.408 (0.234–0.714)]	0.8904 [1.035 (0.632–1.695)]	0.3445 [1.241 (0.793–1.941)]	0.0009 [1.903 (1.296–2.794)]			

Significant differences between the case and control groups are italicized

OR odds ratio, CI confidence interval, AS asthenozoospermia, OAS oligoasthenozoospermia, Oligo oligozoospermia, NOA non-obstructive azoospermia

asthenozoospermia ($p = 0.0012$), while it was a risk factor for non-obstructive azoospermia patients ($p = 0.0009$) (Table 5).

Potential biological functions of *DJ-1* promoter Ins/Del gene polymorphisms

The *DJ-1* gene polymorphisms g.-6_+10del (rs901561484) and g.168_185del (rs373653682) were scanned for transcriptional start sites (TSS) and core promoter elements by EPD and ElemeNT. As shown in Fig. 2, in silico analysis of these indel gene polymorphisms revealed several potential binding sites for transcription factors such as AhR, WT1 I, NF-1, and Nrf2 for the sequences of Ins (I) allele of *DJ-1* g.-6_+10del (Fig. 2(A)) and also Elk-1, NF-1, and Nrf2 transcription factors for sequences of Ins allele of *DJ-1* g.168_185del (Fig. 2(B)). Furthermore, the Dup allele of *DJ-1* g.168_185del with a duplicated Ins sequence includes more binding site elements than the one-repeat allele and also contains a specific *cis* element for transcription factors such as E1aE-A, YY1, DBP, and EBF (Fig. 2(B')).

The prediction of the core promoter elements via ElemeNT tool shows that the sequences of Ins allele and/or Dup allele of *DJ-1* g.-6_+10del and g.168_185del gene polymorphisms had not contained core promoter element sequences and the existence and/or absence of these sequences does not change the core promoter elements in the promoter region of *DJ-1* gene (data not shown).

Discussion

To the best of our knowledge, the present study was the first demonstration that the *DJ-1* g.-6_+10del and g.168_185del gene polymorphisms are associated with susceptibility to male infertility. The results of our study show that the Del (D) allele carriers of *DJ-1* g.-6_+10del polymorphism are significantly associated with a risk of male infertility in total infertile, asthenozoospermia, and oligoasthenozoospermia patients (Tables 2 and 3). Moreover, the Del (D) allele of *DJ-1* g.-6_+10del polymorphism significantly increased in total male infertile, asthenozoospermia, and oligoasthenozoospermia groups, suggesting that the presence of the Del (D) allele in the *DJ-1* promoter may be a risk factor for male infertility. Besides, the frequencies of different genotypes and the Del allele and Dup allele carriers of *DJ-1* g.168_185del gene polymorphisms were associated with male infertility in total infertile and the four different sub-group patients (Tables 2 and 3). Though the Del allele of *DJ-1* g.168_185del gene polymorphism strongly increased the risk of male infertility in total infertile and all four different sub-group patients, the Dup allele of *DJ-1* g.168_185del gene polymorphism was correlated with increased susceptibility to male infertility in total infertility, oligoasthenozoospermia, and non-obstructive azoospermia, proposing that the mutant alleles of *DJ-*

Fig. 2 Potential binding sites for transcription factors of the Ins (I) allele of DJ-1 g.-6_+10del polymorphism (A) and potential binding sites for transcription factors of the Ins allele (B) and Dup allele (B') of DJ-1 g.168_185del polymorphism

I g.168_185del gene polymorphism, Del allele, and Dup allele might be risk factors for male infertility. Haplotype analysis of DJ-1 g.-6_+10del and g.168_185del polymorphisms revealed that the D-Dup and I-Del haplotype frequencies significantly increased the risk of male infertility in total infertile and different sub-group patients, while I-Ins haplotypes were associated with decreased risk of male infertility in total and sub-group patients, implying that the D-Dup and I-Del haplotypes are risk factors and, also, I-Ins haplotype is a protective factor in male infertility susceptibility in the current study population.

Because of the high polyunsaturated fatty acid content in their plasma membranes, spermatozoa are particularly prone to oxidative damage and strong evidence suggests the intrinsic deficiencies of antioxidant capability have detrimental effects on sperm despite of the number, motility and morphology, and consequently low fertilization rates [6–8]. Therefore, spermatozoa recruit various antioxidant defense systems to protect themselves against ROS damage. DJ-1 was reported to have critical roles in antioxidative stress reactions [19]. Upon oxidative stress, DJ-1 indirectly causes the translocation of the Nrf2 into the nucleus to activate various antioxidative stress genes, including GSTM1 and SOD1, thereby decreasing the ROS level [20]. DJ-1 stimulates the expression of superoxide dismutase (SOD 3) and glutathione ligase genes to reduce the ROS level [21, 22]. Furthermore, DJ-1 stimulates anti-apoptotic gene expression and suppresses apoptosis signal-regulating kinase pathways [15, 23–25]. Thus, the decrease of DJ-1 levels in spermatozoa may result in enhanced oxidative stress, sperm damage, deformity, and ultimately male infertility.

The DJ-1 g.-6_+10del and g.168_185del gene polymorphisms, rs901561484 and rs373653682, are located in the promoter region and could affect the transcriptional activity of this gene. The g.168_185del variant is potentially functional due to its location 168 bp after the transcriptional activation site and approximately 50 bp downstream of an SP1 binding site [35]. Moreover, it has been shown that the g.-6_+10del variant reduces expression by approximately 50% [46]. In the current study, the Del (D) allele and the Del (D) allele carriers of DJ-1 g.-6_+10del polymorphism significantly increased the risk of total male infertile, asthenozoospermia, and oligoasthenozoospermia patients, suggesting that the presence of the Del (D) allele in DJ-1 promoter may be a risk factor for male infertility. Besides, the genotype and allele frequencies of DJ-1 g.168_185del gene polymorphisms were associated with male infertility in total infertile and four different sub-group patients, suggesting that the mutant alleles of DJ-1 g.168_185del gene polymorphism and the Del and Dup alleles might be risk factors for male infertility.

Since genetic variants in the promoter region can affect gene transcription activity via altering the DNA-binding ability of transcription factors, we consequently evaluated the potential biological functions of DJ-1 gene polymorphism, g.-6_+10del (rs901561484), and g.168_185del (rs373653682) using bioinformatic analysis. The in silico analysis showed the presence of Ins allele and/or Dup allele DJ-1 g.-6_+10del and g.168_185del gene polymorphism could be sequences to bind to more nuclear factors and probably affect transcriptional activity (Fig. 2), but it seems that

extra transcription factor binding sites in mutant Dup allele of DJ-1 g.168_185del polymorphism can lead to transcriptional interference [47]. The over-activity of *DJ-1* in wild Ins alleles of g.-6_+10del and g.168_185del leads to high antioxidant capacity of spermatozoa, which can increase the resistance of spermatogonia to oxidative stress and interfere with their dependent apoptosis and thus increase the likelihood for the development of normal sperm during spermatogenesis.

It has been revealed that DJ-1 plays a critical role in male fertilization modulated with its two different major functions in germ line cells and specific function in sperm. The PIAS (protein inhibitor of activated STAT)α, which is specifically expressed in the testes and which downregulates the transcriptional activity of the androgen receptor (AR), has been identified as a DJ-1 binding protein [48]. DJ-1 directly binds to the androgen receptor binding site of PIASα and antagonizes the suppression activity of PIASα to AR by absorbing PIASα from the AR–PIASα complex, which indicates that DJ-1 is a positive regulator of the AR-dependent transcriptional activity [48]. These results suggest that DJ-1 affects the production of sperm by activating the AR to express male-specific genes. Besides, it has been shown that DJ-1 was located on the surface of the posterior part of the head, the anterior part of the midpiece, and spermatozoa flagella and anti-DJ-1 antibodies were capable of significantly inhibiting in vitro fertilization of both zona-intact and zona-free hamster oocytes, suggesting that DJ-1 plays two distinct roles in fertilization, binding to the egg process and flagellar movement [32–35]. Overall, DJ-1 affects the production of the sperm by activation of the AR to express male-specific genes, and it has an important role in the flagellar movement and the fertilization function of mature sperm. Therefore, the downregulation of the DJ-1 protein could affect the sperm production process resulting in a decreased amount of sperm and/or could then affect the viability and/or motility of sperm and/or both. Interestingly, it has been reported that the levels of DJ-1 in asthenozoospermia infertile patients are significantly lower than fertile men [40]. Moreover, it has been shown that DJ-1 differentially expressed in patients with varicocele when compared with control men [49–51]. The results of the current study show that genetic variation in the promoter region of *DJ-1*, g.-6_+10del (rs901561484) and g.168_185del (rs373653682), is associated with male infertility risk in total idiopathic male infertility and four different sub-groups, deficiencies in movement and/or amount of sperm, the asthenozoospermia, oligoasthenozoospermia, oligozoospermia, and non-obstructive azoospermia patients as well.

The limitations of the present study were the lack of comparison of *DJ-1* gene expression in the semen of fertile and infertile men with different genotypes of g.-6_+10del and g.168_185del polymorphisms and, also, the low number of the oligoasthenozoospermia patients. Therefore, further studies using a larger sample size are suggested to determine the effects of these polymorphisms on *DJ-1* gene expression.

In conclusion, our study presents the evidence of a strong association between functional polymorphisms of the *DJ-1* promoter, g.-6_+10del (rs901561484), and g.168_185del (rs373653682) with the risk of male infertility in total idiopathic male infertility and four different sub-groups. In addition, the haplotype-based association analysis suggests the D-Dup and I-Del haplotypes as risk factors as well as I-Ins haplotype as a protective factor in male infertility susceptibility in the current study population. However, functional study and further independent large-scale studies in diverse ethnic populations are warranted to clarify the general validity of our finding.

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Compliance with ethical standards Informed consent was obtained from all subjects, and the study protocol was approved by the ethics committee of the University of Mazandaran and conducted in accordance with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no competing interests.

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