Original Article

Correlation of Novel Single Nucleotide Polymorphisms of *USP26*, *TEX15* and *TNP2* Genes with Male Infertility in North West of Iran

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Abstract

Background: Observational studies that inspected the association of *USP26*, *TEX15*, and *TNP2* novel single nucleotide polymorphism (SNP) with odds of male infertility are sparse. Male infertility prevalence in Iran is reported more than global prevalence, while about 30-50% of infertile male have no distinct reason yet and they are considered as idiopathic male infertility. This study was conducted to investigate association of different SNPs of *USP26*, *TEX15*, and *TNP2* genes with male infertility among the Iranian population.

Materials and Methods: In this population-based case-control study, 120 diagnosed idiopathic azoospermia or severe oligospermia infertile cases range of 25-45 years old, and 120 age-matched fertile controls were recruited. Overall, six different variants from three genes were genotyped including *USP26 rs61741870*, *USP26 rs144039408*, *TEX15 rs323344*, *TEX15 rs61732458*, *TNP2 rs11640138* and *TNP2 rs199536093* by using amplification-refractory mutation system polymerase chain reaction (ARMS-PCR) methods.

Results: Although there was no significant association of *USP26* gene variants (rs61741870 and rs144039408) with men infertility, we found a significant association of *TEX15 rs323344* T allele and odds of idiopathic azoospermia compared to recessive allele (odds ratio [OR]: 0.259, confidence intervals [CI]: 0.083-0.811). We determined significant associations of *TEX15 rs61732458* AC and CA+AA with male infertility compared to normal homozygote (OR: 3.776, CI: 2.049-6.957, OR: 3.818, CI: 2.077-7.016, respectively). Significant association was seen among *TNP2* rs199536093 GG genotype and idiopathic azoospermia compared to normal homozygote (OR: 0.348, CI: 0.129-0.939). We also observed heterozygote overdominance in *TEX15* rs61732458 and *TNP2* rs199536093.

Conclusion: We found novel polymorphisms related to male infertility among Iranian population. However, larger studies are needed to confirm the obtained results.

Keywords: Case-Control, Male Infertility, *TEX15*, *TNP2*, *USP26*

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Introduction

Infertility is one of the major public concerns that affects 15% of young couples trying to conceive worldwide (1, 2). Although male infertility range is not accurately reported, due to cultural differences especially in patriarchal societies, roughly 50% of infertility results from male factors (1, 3). Region-specific reports indicated that prevalence of clinical and epidemiological infertilities among the Iranian population were respectively 20.2 and 12.8%, while the male factors contribute to 34% of total infertility (4, 5).

Given the high prevalence rate and lack of comprehensive data on male infertility risk factors, understanding possible contributors are of great priority to reach better therapeutic strategies. Several contributors are associated with male infertility risk including environmental, lifestyle-related variables, diet, smoking, alcohol consumption, genetic, etc. (2, 3, 6). Genetic causes have long been focused at the heart of contributing factors to male infertility. Various chromosomal aberrations, Y chromosome micro-deletions, gene mutation, and single nucleotide polymorphisms (SNPs) have been individually linked to the risk of male infertility (7). Although intense researches on identifying genes involve in male infertility are available, data on the armamentarium of SNPs are extremely limited. It has been shown that chromosome X-linked genes are significantly related to male infertility, because men are hemizygous for X-linked genes (as they have only one copy number of the X chromosome), and

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these genes are more prone to rapidly evolve in exposure to selective pressure (8).

Large numbers of these genes are associated with sperm and testis, like Ubiquitin-specific protease 26 (*USP26*). *USP26* has only one exon located at Xq26.2 that contributes to the spermatogenesis process included removing histones, regulation of protein turnover during meiosis, germ cell apoptosis, proliferation, and differentiation of spermatogonial stem cells during mitosis (7, 9-11). To date, more than twenty polymorphisms have been reported in *USP26* gene. According to the study performed by Zhang and his colleagues, some of these mutations, including a cluster haplotype (370-371insACA, 494T>C, and1423C>T) are strongly related to male infertility (12). However, results of the other researches did not confirm such associations. Although a recent meta-analysis study reported an association between USP26 gene polymorphisms and male infertility (8), the data are highly contradictory, which may be due to differences in the study population. The other important gene involved in different stages of spermatogenesis is testis expressed 15 (*TEX15*), which is revealed to have a relation with male infertility. This gene is mapped on chromosome 8p12 (13). Recent pieces of evidences indicated that absence of TEX15 led to infertility only in male due to meiotic arrest, meiotic recombination, and disturbance of spermatogenesis; whereas mutant females were fertile (14).

Based on preliminary studies, loss of function mutant of *TEX15* relates to meiotic recombination failure and DNA repair system malfunction. This protein is extremely expressed in post-meiotic germ cells, spermatogonia, and early spermatocytes of mammals and fish (14-16). However, limited region-specific data are available on SNPs of *TEX15* in infertile men. It has been demonstrated that some complex morphological, biochemical, and physiological modifications including removal of histones and DNA condensation occur during spermiogenesis. Formation of mature spermatozoa needs specific events, mediated by spermiogenesis-specific gene products (17-20). Recent studies have shown that transition nuclear protein (*TNP2*) is critical for sperm maturation and fertilization. This gene is located on human chromosome 16p13.3 (21). Premature gene expression of *TNP2* contributed to defective spermatid morphology and male infertility in mice. A recent study reported that family of SNPs including TNP2 (T1019C), (G1272C) and (G del in 1036 and 1046 bp) have no association with idiopathic oligospermia, and azoospermia among the Iranian population (22). By contrast, Iranian men who have varicocele were more likely to have a CC genotype of g. IVS1-26 G > C SNP in TNP2 (21). Given the high prevalence of male infertility, understanding the main genetic contributors, especially SNPs may provide effective therapeutic strategies. Since the data in this field is limited and contradictory, it seems that more studies are needed to reach a reliable result on association of *USP26*, *TEX15*, and *TNP2* polymorphisms with male infertility. This project was conducted to investigate association of different SNPs of *USP26*, *TEX15*, and *TNP2* genes with idiopathic male infertility among the Iranian population using amplification-refractory mutation system polymerase chain reaction (ARMS-PCR).

Materials and Methods

Participants

This project was a case-control study, performed on infertile men with range of 25-45 years old who were recruited to the study from the infertility treatment center of Valiasr hospital (Tabriz, Iran) from March 2018 to July 2019. All cases were diagnosed and confirmed with idiopathic azoospermia or severe oligospermia (sperm count below 5×10^6/ml) by a specialist. The sample size was calculated based on random case-control method, using the following formula:

$$n= (r+1/r)\ (\bar{p}(1-\bar{p})\ (Z_{\beta}-Z_{\alpha/2})\ 2/(p_1-p_2)_2$$

$$n= 2\times(0.29\times(1-0.29)\times(0.84+1.96)^2/(0.2-0.38)^2=95$$

Odds ratio (OR) of exposure in cases relative to controls was considered 2.5. Probability of exposure was 0.38 among the case group (p_2) and 0.2 among the control group (p_1). Ratio of controls to cases (r) was 1. Variability ($\bar{p}$) was considered similar to standard deviation (0.29). The study power was considered 80%, and level of significance was considered 0.05 (23). The minimum sample size required for this study was 95 people per group. However, more participants were included: 120 infertile men as cases, and 120 age-matched, fertile healthy volunteer men as controls. Controls were healthy men with normal semen tests who had at least one child at the participation time. All eligible subjects gave their written informed consent to participate in the project. To determine quality and quantity of sperm samples, semen analysis was performed twice for all participants using microscopic examination methods according to the world health organization (WHO) standard values (24). A 5 ml venous blood sample was taken from each participant, followed by DNA isolation. To exclude other potential causes, all infertile subjects were assessed for physical examination, and all necessary hormonal tests (luteinizing hormone [LH], follicle-stimulating hormone [FSH], testosterone and prolactin), in addition to genetic tests including karyotyping, microdeletion of Y chromosome, and CFTR mutations.

All ethical principles, national norms, relevant instructions, and rules related to conducting medical research in Iran have been observed in this project. All participants performed the written consent. The project was approved by the Science and Research Branch of Tehran`s Islamic Azad University's Ethical Committee (Tehran, Iran, IR.IAU.SRB.REC.1398.001).

Extraction of peripheral blood DNA

Five ml of peripheral blood was taken from each individual for genomic DNA extraction and stored at -80°C. Genomic DNA was extracted from the blood using PCR-BIO Rapid Extract PCR Kit (from PCR Bio Systems

Ltd., UK) according to the instructions mentioned in the kit brochure. Concentration and quantity of the extracted DNA were measured at 260 nm and 280 nm (A260/280) using a NanoDrop 2000 spectrophotometer (Denovix Ds-11 spectrophotometer).

Genotyping

Allele-specific polymerase chain reaction PCR or ARMS method was used to identify variants of gene' polymorphisms. In order to proliferate our targeted genes, all primers were designed using primer 3 software (the software Gene Runner version 3.05 (Hastings, USA). Quality and specificity of primers were analyzed using Oligo Analyzer software and primer blast on the NCBI website. We used a ready-to-use mastermix to amplify each gene (PCR Biosystems Ltd., UK). PCRs were performed with a final volume of 25 µl. PCR products were observed in 2% gel electrophoresis using Novel juice stain (Cat. No.LD001-1000, GeneDirex, Taiwan). Samples were genotyped again to validate the obtained results.

Primers and cycling conditions

Cycling conditions for all primers were as follows: 95˚C for 1-3 minutes for the first cycle, followed by 29-40 cycles of denaturation at 95˚C for 30-40 seconds, optimum annealing temperature for each reaction (Table 1) and extension at 72˚C for 1 minute. Ultimately, final extension was applied at 72˚C for 5 minutes. PCR conditions were the same for mutant and wild-type reactions. Sequences of primers are summarized in Table 1.

Statistical analysis

For each polymorphism, the Hardy-Weinberg equilibrium (HWE) was evaluated in case and control groups using chi-square test (χ^2). In order to examine whether HWE in a group is disrupted or not, obtained χ^2 was compared to the statistic table. If it was bigger than the number in the statistics table (3.8), HWE will be disrupted in that group unless the group is in HWE. Association of SNP with heterozygote over-dominance of *USP26*, *TEX15*, and *TNP2* with male infertility was assessed by using genetic models or conditional logistic regression. If the control group was under HWE, but the case group was not, a recessive/dominant pattern will be used. If both the case and control groups were in HWE, the multiplicative pattern will be used for statistical analysis. Additive model will be used when none of the case or control group was in HWE or the case group was under HWE but the control group was not. Statistical analyses were performed using SPSS version 22.0, statistical software (IBM, USA). $P<0.05$ were considered statistically significant.

Table 1: Polymerase chain reaction (PCR) amplification primers

Type of primer	Primer sequence (5´-3´)	Annealing temperature	Productsize (bp)
USP26 A>G (rs61741870)			158
Common reverse primer	TCT AGC ACA ACA CAG AAG GA		
Wild type forward primer	AAC TCT CCG CAA GTA AGT GTA A	54°C for 15 seconds	
Mutant type forward primer	AAC TCT CCG CAA GTA AGT GTA G	55°C for 15 seconds	
USP26 G>C (rs144039408)			242
Common reverse primer	TAC CTC TCC ATC AAC CTT CC		
Wild type forward primer	TTG CAA ATG ACT TCC TC	53°C for 30 seconds	
Mutant type forward primer	TTG CAA ATG ATG ACT TCC TG	53°C for 30 seconds	
TEX15 T>G (rs323344)			198
Common reverse primer	ATC AAT TAC CTG AAG AGC ACT G		
Wild type forward primer	GTC CAA AGT ACA GTA TTC CTC AGT	57°C for 40 seconds	
Mutant type forward primer	CAA CAA GTA CAG TAT TCC TCA GG	57°C for 40 seconds	
TEX15 C>A, (rs61732458)			268
Common reverse primer	ATC TTT CCA CGT TAT TCT GTC C		
Wild type forward primer	TCA GAC CTG TAG AGA AAA AGA GC	57°C for 40 seconds	
Mutant type forward primer	GCA GAG CTG TAG AGA AAA AGA GA	57°C for 40 seconds	
TNP2 C>T (rs11640138)			187
Common reverse primer	ACA TTC ACC TAG CCA ACT GC		
Wild type forward primer	GGA GTA CAA AAC CAA GAC GC	57.5°C for 40 seconds	
Mutant type forward primer	AGG AGT ACA AAA CCA AGA CGT	57.5°C for 40 seconds	
TNP2 C>T (rs199536093)			214
Common reverse primer	ACT TCA ACC CAA CTG GAG C		
Wild type forward primer	TTG TAC ACC TGC TGG ATC C	56.5°C for 40 seconds	
Mutant type forward primer	TTG TAC ACC TGC TGG ATC G	56.5°C for 40 seconds	

Results

Semen analysis parameters are summarized in Table 2. The allelic and genotypic frequencies of *USP26*, *TEX15*, and *TNP2* variants in fertile and infertile men are presented in Table 3. We identified a total of six variants for three genes (*USP26*, *TEX15* and *TNP2*). There was no significant difference between the groups in terms of *USP26* variants and *TPN2* rs11640138 variant. There were significant differences between the cases and controls, regarding *TEX15 rs323344*, and rs61732458 variants as well as *TNP2* rs199536093 variant.

Associations of the three gene polymorphisms with male infertility are shown in Table 4. There was no significant associations between *USP26* gene variants (rs61741870 and rs144039408) and male infertility (see ORs and 95% confidence intervals [CIs]). Examination of HWE indicated that the equilibrium for *USP26 rs61741870* (*A>G*) was disrupted in both cases and controls (χ^2=19.3498 and χ^2=15.3338, respectively). In terms of rs144039408 (G>C), none of the case or control groups were in HWE (χ^2=51.8283 and χ^2= 45.4398, respectively). So the additive model was used for statistical analysis.

Table 2: Semen characteristic

Parameter	Fertile controls (n=120)	Infertile cases (n=122)	Lower reference limit WHO 2010
Volume (ml)	2-8.5	1-8	1.5
Total sperm count in ejaculate ($\times10^6$ ml)	50-120	<10	39
Sperm count ($\times10^6$/ml)	35-95	0-5	15
Total motility (%)	45-85	-	40 (38-42)
Progressive motility (PR), %	42-80	-	32 (31-34)
Vitality (%)	55-65	-	58 (55-63)
Normal morphology (%)	4-5	0-1	3-4
PH	7.2-8	7.2-7.6	>7.2
Viscosity	N	N- M-H	N (smooth and watery)
Liquefaction time	20-5 minutes	20-45 minutes	<60
Round cells ($\times10^6$/ml)	0-4	0-4	<5
Sperm agglutination	0-1	0-1	<2

N; Normal, M; Moderate, and H; High.

Table 3: Distribution of the *USP26*, *TEX15*, and *TNP2* genotypes in infertile patients and controls

Genotype	MAF (ALFA)	Case (n=122)	Control (n=120)	P value*
USP26 61741870 AA	<=0.007022/677	20 (16.7)	28 (23.3)	0.367
USP26 61741870 AG/AA		84 (70.0)	80 (66.7)	
USP26 61741870 GG		16 (13.3)	12 (10.0)	
USP26 144039408 GG	C=0.000448/5	57 (46.7)	45 (37.5)	0.341
USP26 144039408 GC/GG		21 (17.2)	23 (19.2)	
USP26 144039408 CC		44 (36.1)	52 (43.3)	
TEX15 323344 TT	G=0.148548/20628	49 (40.5)	48 (40.0)	0.042
TEX15 323344 GT/TT		68 (56.2)	58 (43.3)	
TEX15 323344 GG		4 (3.3)	14 (11.7)	
TEX15 61732458 CC	A=0.000446/46	19 (15.6)	50 (41.3)	0.000
TEX15 61732458 AC/CC		99 (81.1)	69 (57.0)	
TEX15 61732458 AA		4 (3.3)	2 (1.7)	
TNP2 199536093 CC	G=0.000089/1	22 (18.2)	23 (19.2)	0.007
TNP2 199536093 CG/CC		91 (72.5)	73 (60.8)	
TNP2 199536093 GG		8 (6.6)	24 (20.0)	
TNP2 11640138 CC	T=0.495608/94782	4 (3.3)	6 (5.0)	0.811
TNP2 11640138 CT/CC		110 (91.7)	108 (90.0)	
TNP2 11640138 TT		6 (5.0)	6 (5.0)	

Data are presented as n (%).*; P values were obtained from χ^2 test and MAF; Minor allele frequency. Mentioned MAF is according to the results of ALFA project.

There was a significant association between *TEX15* rs323344 T allele (recessive allele) and odds of idiopathic azoospermia compared to the dominant allele (P=0.014, OR: 0.259, CI: 0.083-0.811). Since the OR for this polymorphism was less than one, it can be indicated that has a protective effect on fertility and it may reduce the rate of infertility. The HWE examination test for *TEX15* rs323344 (T>G) showed under HWE for the control group, while it was not observed in the case group (χ^2 = 0.3126 and χ^2=11.21, respectively). Therefore, these genotypes showed a recessive/dominant pattern. In terms of rs61732458, there was significant associations between rs61732458 AC genotype as well as CA+AA genotype with male infertility compared with normal homozygote (P=0.000, OR: 3.776, CI: 2.049-6.957; P=0.000, OR: 3.818, CI: 2.077-7.016, respectively). For rs61732458 (C>A), HWE was disrupted in two study groups, as well (χ^2 =51.2064 and χ^2=15.1195, respectively).

We found a significant association among *TNP2* rs199536093 GG genotype and idiopathic azoospermia compared to normal homozygote (P=0.034, OR: 0.348, CI: 0.129-0.939). According to the obtained OR, this genotype has also a protective effect on fertility. There was no significant association between other *TNP2* rs199536093 genotypes as well as rs11640138 genotypes with men infertility in our study population. For TNP2 rs11640138 (C>T), none of the cases or controls were in HWE (χ^2=83.4352 and χ^2=76.8, respectively). Such trends were seen for *TNP2* rs199536093 (C>G) regarding the HWE in the case and control groups (χ^2=33.2924 and χ2=5.6377, respectively). We also analyzed the results to investigate heterozygote overdominance. Obtained results indicated that there was over dominance in variants *TEX15* rs61732458 and *TNP2* rs199536093 but not in the others. Results are summarized in Table 4.

Table 4: Genetic models of single nucleotide polymorphism (SNP) associated with male infertility

Genotype	OR (95%CI)	P value
USP26 61741870 AA	1.00	
USP26 61741870 AG/AA	1.470 (0.767-2.817)	0.244
USP26 61741870 GG	1.867 (0.727-4.794)	0.192
USP26 61741870 AG+GG	1.522 (0.803-2.886)	0.197
USP26 61741870 AG/AA+GG	1.167 (0.677-2.011)	0.579
USP26 144039408 GG	1.00	
USP26 144039408 GC/GG	0.721 (0.355-1.465)	0.365
USP26 144039408 CC	0.668 (0.381-1.170)	0.157
USP26 144039408 GC+CC	0.684 (0.410-1.143)	0.146
USP26 144039408 GC/GG+CC	0.877 (0.456-1.686)	0.694
TEX15 323344 dominant (G)	0.98 (0.585-1.639)	0.937
TEX15 323344 recessive (T)	0.259 (0.083-0.811)	0.014
TEX15 61732458 CC	1.00	
TEX15 61732458 AC/CC	3.776 (2.049-6.957)	0.000
TEX15 61732458 AA	5.263 (0.890-31.137)	0.068
TEX15 61732458 CA+AA	3.818 (2.077-7.016)	0.000
TEX15 61732458 AC/CC+AA	3.244 (1.818-5.789)	0.000
TNP2 199536093 CC	1.00	
TNP2 199536093 CG/CC	1.303 (0.673-2.523)	0.431
TNP2 199536093 GG	0.348 (0.129-0.939)	0.034
TNP2 199536093 CG+GG	1.067 (0.558-2.040)	0.844
TNP2 199536093 CG/CC+GG	1.953 (1.125-3.392)	0.017
TNP2 11640138 CC	1.00	
TNP2 11640138 CT/CC	1.528 (0.149-5.565)	0.518
TNP2 11640138 TT	1.55 (0.275-8.189)	0.691
TNP2 11640138 CT+TT	1.526 (0.420-5.552)	0.518
TNP2 11640138 CT/TT+CC	1.222 (0.507-2.947)	0.655

OR; Odds ratio and CI; Confidence intervals.

Discussion

In this population-based, case-control study, we found a direct association between *TEX15* rs323344 T allele, and *TEX15* rs61732458 AC and CA+AA genotypes with idiopathic azoospermia cases risk. Such a relationship persisted in *TNP2* rs11640138 GG genotype with odds of male infertility. We failed to find any significant association between *USP26* gene variants and risk of infertility among our study population. As far as we are aware, this is the first observational study exploring association of specific variants of *USP26*, *TEX15*, and *TNP2* genes with risk of male infertility among Iranian population.

SNPs of the genes involved in male infertility remained an elusive issue. Recently, *USP26* has been considered as a main mediator of spermatogenesis in men with non-obstructive azoospermia (9, 25). However, in the present study, we failed to find a significant relationship between *USP26* gene variants and male idiopathic azoospermia. These variants were not examined previously. However, our findings were in accordance with the recent meta-analysis results, indicated that some variants of *USP26* including c.363_364insACA, c.494T>C, c.1423C>T, c.1090 C>T and c.1737 G>A were not related to male infertility (12). Despite this, some variants such as 576G>A are potentially related to sperm motility, but not directly associated with human sperm count (7). Our results were in contrast with previous reports indicating the role of *USP26* gene polymorphisms in men infertility among Arabs, Taiwan, and Caucasian population (11, 26, 27). Mutations in *USP26* gene including 370-371insACA, 1423C>T ,and 494 T>C are related to male infertility among the Iranian population (10). The variations c.576G>A and 1090C>T are also related to male azoospermia (11, 27). It should be mentioned that our studied *USP26* variants are generally different from the other studies. Moreover, azoospermia is in association with environmental factors, origin of the study population as well as the age of participants. These differences could justify the controversial results.

Based on the strong evidences, expression of TEX15 contributes to regulation of meiosis in germ cells in both testis and ovary (28, 29). Mutations of *TEX 15* gene lead to meiotic recombination failure and consequently disruption of spermatogenesis. We realized that *TEX15* rs323344 T allele (recessive allele) associates with odds of idiopathic azoospermia. In addition, we reached a significant association between rs61732458 AC genotype as well as CA+AA genotype and male infertility. Our findings were in line with others reporting significant association between *TEX15* single nucleotide polymorphisms and male infertility (13, 15, 16, 30). TEX15 rs323346 and rs323347 are considered as risk factors for male infertility among the Chinese Han population (16). It has been shown that nonsense mutation and single nucleotide deletion in TEX15 (c.2419A>T, p.Lys807*, and c.3040delT, p.Ser1014Leu fs*5) contributed to non-obstructive azoospermia (13, 30). By contrast, the study of Plaseski and his colleagues on individuals of different ethnic origins including Macedonians, and Albanians did not find any significant association between *TEX15* polymorphism and male infertility (31). As mentioned, the variants studied in this project were completely different from the other studies. Therefore, it could lead to some inconsistency.

TNP2 is among the genes that are frequently studied for infertility concept. This protein is involved in the establishment of species-specific sperm nucleolus morphology (19). This is the first report demonstrating a significant association between rs199536093 GG genotype and risk of idiopathic azoospermia among the Iranian population. Our results were in agreement with other reports from the Iranian population which demonstrated an association of *TNP2* rs8043625 variant with varicocele risk in men (21). Similarly, the other reports have shown SNPs in the open-reading frame of *TNP2* gene or deletion on *TNP2* gene which leads to male infertility (32, 33). By contrast, the study of Siasi and his colleagues indicated that SNPs in TNP2 (G1272C) were not related to idiopathic azoospermia and male infertility in the Iranian population (22). It should be mentioned that type of SNPs in the previous studies were different from those studied in our project. Therefore, it could result in controversial findings.

USP26, *TEX12* and *TNP2* are among genes that contribute to essential stages of fertility. These potential roles can be explained by various mechanisms. They are involved in pre- and post- meiotic stages of mammalian spermatogenesis (7), chromosomal synapsis, and meiotic recombination of germ cells (14). They are necessary for maintaining the normal processing of protamines and completion of chromatin condensation during meiosis (19, 20).

This project was the first study to investigate the relationship between novel variants of *USP26*, *TEX15*, *TNP2* genes and odds of infertility among the Iranian men, which was the strength of this study. However, some limitations also need to be considered in interpretation of the findings. Some confounders including environmental factors, smoking, high-risk jobs, etc. were not taken into account. Therefore, the obtained results should be generalized cautiously.

Conclusion

This study suggests some novel polymorphisms as the predisposition of male infertility in idiopathic cases among the Iranian population, which was not considered before. However, the results should be validated by studies in different populations.

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Authors' Contributions

E.Gh., S.A.A.; Equally made contribution to conception, DNA extraction, SNP genotyping, analysis and interpretation of data. M.Kh., M.R.M.; Contributed in data analysis and writing the manuscript. All authors read and approved the final manuscript.

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