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Association of Novel Single Nucleotide Polymorphisms of Genes Involved in Cell Functions with Male Infertility: A Study of Male Cases in Northwest Iran

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Abstract

Background: Infertility is a global health problem caused by various environmental and genetic factors. Male infertility accounts for 40–50% of all cases of infertility and approximately half of them are grouped as idiopathic with no definitive causes. Previous studies have suggested an association between some SNPs and infertility in men. In this study, an attempt was made to investigate the association of 7 different SNPs of 4 genes involved in common cell functions with male infertility.

Methods: MTHFR rs1801131 (T>G), MTHFR rs2274976 (G>A), FASLG rs80358238 (A>G), FASLG rs12079514 (A>C), GSTM1 rs1192077068 (G>A), BRCA2 rs4987117 (C>T), and BRCA2 rs11571833 (A>T) were genotyped in 120 infertile men with idiopathic azoospermia or severe oligospermia and 120 proven fertile controls using ARMS-PCR methods. Next, 30% of SNPs were regenotyped to confirm the results. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using SPSS statistical software to evaluate the strength of association. The $p<0.05$ were considered statistically significant.

Results: Statistical analysis revealed significant association between MTHFR rs2274976 AA variant (OR: 10.00, CI: 3.203-31.225), FASLG rs12079514 AC variant (OR: 0.412, CI: 0.212-0.800), and BRCA2 rs11571833 TT variant OR: 6.233, CI: 3.211-12.101) with male infertility, but there was no significant difference between case and control groups in MTHFR rs1801131 (p= 0.111), GSTM1 rs1192077068 (p=0.272), BRCA2 rs4987117 (p=0.221), and FASLG rs80358238 (p=0.161).

Conclusion: Our findings suggested that some novel polymorphisms including MTHFR rs2274976, FASLG rs12079514, and BRCA2 rs11571833 might be the possible predisposing risk factors for male infertility in cases with idiopathic azoospermia.

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Introduction

Infertility is one of the health problems in the world with almost 15-20% prevalence among couples at childbearing age (1, 2). According to WHO, couples are considered infertile who are unable to get spontaneous pregnancy after a year of unprotected regular intercourse (3). Male infertility accounts for 40–50% of all cases of infertility and approximately half of them are grouped as

idiopathic with no definitive causes (4, 5). Several congenital and acute risk factors have been reported as the causes of infertility problems. Among them, genetic abnormalities with the reported rate of 15% are mostly related to sperm count (6-8). Approximately 1,000 genes are involved in the sperm production process, *i.e.* spermatogenesis. Any defect in each of these genes can lead to spermatogenesis impairment. Although the role of some genetic variants in spermatogenic failure has been proven and their role as good targets for diagnostic tests has been implicated, the function of many others is still unclear (9, 10).

According to previous reports, about 50 percent of male infertility cases are related to oxidative stress (11). Reactive oxygen species (ROS) can lead to infertility in men by directly damaging sperm DNA or reducing sperm motility and subsequently its permeability to the oocyte (4, 12, 13). ROSs are products of cellular metabolism formed by enzymatic reduction of oxygen during the energy production process (14). There are two distinct supergene families to encode cytosolic and membrane-bound forms of glutathione S-transferase (GST) (15). The cytoplasmic form of GST is classified into eight groups of alpha, kappa, mu, omega, pi, sigma, theta, and zeta. GSTM1 located on chromosome 1p13.3 is classified in the mu class of GST with the main role of oxidation protection and detoxification of ROSs (16). Several polymorphisms have been identified in this gene, which suggests that different variants of this metabolic enzyme may affect its function as well.
Gene variation in the methylenetetrahydrofolate reductase (MTHFR) is another risk factor that has been associated with a variety of diseases, ranging from meiotic nondisjunction of chromosomes in Down syndrome to male infertility (17). MTHFR is a critical regulatory enzyme in folate metabolism, which is located on the short arm of chromosome 1 (1p36.3). Folate is essential for DNA synthesis and methylation (18, 19). Methionine, an essential amino acid, acts as a universal methyl donor in its activated form, named SAM [1]. By methyl group donation of SAM, S-adenosylhomocysteine is formed which can subsequently be hydrolyzed to homocysteine and adenosine. Methionine synthase (MTR) can remethylate homocysteine to methionine. This reaction is mediated by 5-methyl-tetrahydrofolate (CH3-THF) as a methyl donor and vitamin B12 as a cofactor. MTHFR catalyzes the formation of CH3-THF by reduction of 5, 10-methylenetetrahydrofolate (20). There is great interest in studying the association of polymorphisms of the MTHFR gene with a variety of diseases, including male infertility. Significant association of the rs1801133 (c.677C>T) variant, a major functional polymorphism of this gene, with male infertility is reported in previous studies (21) but results are inconsistent regarding rs-1801131 (1298 A>C) and research is scare about many other SNPs (8).

Programmed cell death known as apoptosis, is an evolutionarily conserved mechanism that plays an important role in eliminating defective or unwanted cells (22, 23). Apoptosis occurs frequently during spermatogenesis and 25-75% of spermatogonia are destroyed during apoptosis before they reach maturity (23, 24). The testis has a predominant expression of FAS and FASLG which are major regulators of apoptosis during spermatogenesis (25). Interaction between FAS and its natural ligand, FASLG, triggers the intrinsic apoptotic pathway. FAS-induced apoptotic cascade continues by various caspases including caspase-8 (26). In some cases, abortive apoptosis sometimes happens. In this process, apoptosis begins but remains incomplete and produces cells with apoptotic features including fragmented DNA and membrane-bound FAS (27). Previous studies have suggested a correlation between the genes encoding these proteins and semen quality which makes them important candidates for male infertility studies. Correlation between FAS-670A/G (rs1800682: A.G) and CASP8-6526N ins/del (rs-3834129:–/C TTACT) polymorphisms with decreased normal sperm concentration and low apoptosis rate are reported in previous studies (23, 28).

BRCA2 is considered as another candidate gene for male infertility in this paper. BRCA2 is a DNA repair-associated gene that is located on chromosome 13q13.1 (29). This 3418 amino acid protein contains several conserved motifs with 70 amino acids known as BRC motifs which help RAD51 recombinase to react with DNA during DNA repair (30). As the BRCA2 gene is expressed highly during meiosis and spermatogenesis (30-32), it seems that BRCA2 variants may alter or arrest meiosis and lead to defective spermatogenesis causing infertility. Studies indicate the association of BRCA2 variants with breast and ovarian cancer, but the role of these polymorphisms in male infertility is still unclear. In this

1. S-adenosylmethionine

study, two SNPs of this gene (rs11571833 and rs4987117) were selected to investigate their association with male infertility in cases with idiopathic infertility. The rs11571833 produces a stop codon inside the coding sequence and consequently leads to truncated protein production. This SNP is reported clinically significant in breast cancer. The rs4987117, a missense that replaces the threonine (T) by methionine (M), may influence the proper function of the protein. The clinical significance of this SNP is uncertain.

In this study, the association of 7 SNPs in genes with common functions in cells including FASLG, GSTM1, BRCA2, and MTHFR with male infertility was investigated among Iranian men.

Methods

Sample: In this study, 120 infertile men with idiopathic azoospermia or severe oligospermia (Sperm count below $5\times10^6/ml$) in the age range of 25-45 years at infertility treatment center of Tabriz Valiasr Hospital and 120 fertile men of the same age group with at least one child voluntarily participated in this study and their informed consent was obtained. All participants' semen was analyzed twice by microscopic examination according to WHO standard values published in 2010. Since some factors including hormonal disorders of LH, FSH, prolactin, and testosterone, numerical chromosome abnormalities, microdeletions of chromosome Y, CFTR mutations, and varicocele are considered as routine causes of male infertility, the patients were subjected to physical examination, and all necessary hormonal and genetic tests were performed to exclude these abnormalities. This study was approved by the ethics committee of Islamic Azad University, Science and Research Branch (Approval number: IR.IAU.SRB.REC.1398.001).

DNA extraction: First, 5 *ml* of peripheral blood was taken from each individual for genomic DNA extraction. DNA was extracted by using PCRBIO Rapid Extract PCR Kit (PCR Biosystems, UK). DNA concentration and purity were measured using Nanodrop (Denovix Ds-11 spectrophotometer).

Genotyping: Genotyping of all polymorphisms was determined by allele-specific PCR or ARMS (Amplification refractory mutation syste). All primers were designed using Primer 3 software. The quality and specificity of primers were analyzed using Oligo Analyzer software and primer-BLAST on the NCBI website. PCRs were performed with a final volume of 25 μl. PCR products were separated and observed in 2% gel electrophoresis using Novel juice stain (Cat.No. LD001-1000). Finally, 30% of samples were re-genotyped to confirm the results.

Single nucleotide polymorphisms in MTHFR: The MTHFR T>G(rs1801131) was amplified by using common forward primer 5`-TCT ACC TGA AGA GCA AGT CC-3`, wild type reverse primer 5`-GAA GAC TTC AAA GAC ACT TCT-3`, and mutant type reverse primer 5`-GAA GAC TTC AAA GAC ACT CG-3` with the following cycling conditions for wild type amplification; 95 ℃ for 1 *min* for the first cycle, 95 ℃ for 15 *s*, 47 ℃ for 15 *s*, 72 ℃ for 7 *min* for 40 cycles, and the final extension time at 72 ℃ for 7 *min*. All the cycling conditions for the mutant type were the same except the annealing temperature which was 51.4 ℃. The primers used to amplify MTHFR G>A (rs2247976) were common reverse primer 5`-GGA AGT AGT TGT CGT GGA TG-3`, wild type forward primer 5`-TGC CCT GTG GAT TGA GAG-3`, and mutant type forward primer 5`-TGC CCT GTG GAT TGA GAA-3`, and the cycling conditions were as follows: 95 ℃ for 4 *min* for the first cycle, 95 ℃ for 45 *s*, 55 ℃ for 30 *s*, 72 ℃ for 90 *s* for 34 cycles, and final extension at 72 ℃ for 7 *min*. The size of PCR products was 89 base pairs (*bp*) for both polymorphisms.

Single nucleotide polymorphisms in GSTM1: GSTM1 G>A (rs1192077068) was amplified using common forward primer 5`-GAT CTG GCT GGT GTC TCA AG-3`, wild type reverse primer 5`-TCA CCT AGC GGA TGT CCC-3`, and mutant type reverse primer TCA CCT AGC GGA TGT CCA with the following cycling conditions: 95 ℃ for 5 *min* for the first cycle, 95 ℃ for 30 *s*, 57 ℃ for 30 *s*, 72 ℃ for 1 *min* for 30 cycles, and final extension at 72 ℃ for 5 *min* for both mutant and wild type. PCR products with a size of 393 *bp* were observed in 2% gel electrophoresis.

Single nucleotide polymorphisms in FASLG: FASLG A>G (rs80358238) with the PCR product size of 307 *bp* was amplified by using wild type forward primer 5`-TTT CAG GCA AGT CCA ACT CCA-3`, mutant type forward primer 5`- TTT CAG GCA AGT CCA ACT CCG-3`, and common reverse primer 5`-AGA TTG AAC ACT GCC CCC AG-3` with the following cycling conditions: 95 ℃ for 5 *min* for the first cycle, 95 ℃ for 1 *min*,

57 ℃ for 45 *s*, 72 ℃ for 45 *s* for 30 cycles, and the final extension at 72 ℃ for 7 *min*. PCR condition for both mutant and wild type was the same.

Primers for FASLG A>C (rs12079514) were wild type forward primer 5`-GAT CAA TGA AAC TGG GCT GTA-3`, mutant type forward primer 5`-ATC AAT GAA ACT GGG CTG TC-3`, and common reverse primer 5`-GCA CTG GTA AGA TTG AAC ACT G-3`. PCR condition for both wild and mutant type was one cycle at 95 ℃ for 3 *min* and 30 cycles at 95 ℃ for 30 *s*, 55 ℃ for 30 *s*, and 72 ℃ for 1 *min*. The final extension was at 72 ℃ for 5 *min*. PCR product size was 216 *bp*.

Single nucleotide polymorphisms in BRCA2: Primers used for BRCA2 A>T (rs11571833) were wild type forward primer 5`-CTC CTC AGA CGA CTC CAT TTA-3`, mutant type forward primer 5`-TCC TCA GAC GAC TCC ATT TT-3`, and common reverse primer 5`-GTA CTG GCA TGG GAA CTC TC-3`. PCR conditions for wild type amplification were as follows: 95 ℃ for 5 *min* for one cycle, 95 ℃ for 1 *min*, 56.5 ℃ for 45 *s*, 72 ℃ for 40 *s* for 30 cycles, and final extension at 72 ℃ for 5 *min*. PCR conditions for the mutant type included the first cycle at 95 ℃ for 5 *min*, 35 cycles at 95 ℃ for 45 *s*, 52 ℃ for 35 *s*, and 72 ℃ for 35 *s* and a final extension at 72 ℃ for 5 *min*. PCR product size was 247 *bp*.

BRCA2 C>T (rs4987117) was amplified by using wild type forward primer 5`-TCT AGA TAA TGA TGA ATG TAG CTC-3`, mutant type forward primer 5`-TCT AGA TAA TGA TGA ATG TAG CTT-3`, and common reverse primer 5`-CACA AAC TAA CAT CAC AAG GT-3`. PCR conditions for both wild and mutant types included the first cycle at 95 ℃ for 3 *min*, 30 cycles at 95 ℃ for 30 *s*, 47.5 ℃ for 30 *s*, and 72 ℃ for 1 *min*, and final extension at 72 ℃ for 5 *min*. The PCR product size was 137 *bp*.

Statistical analysis: The frequency of genotypes in case and control groups was evaluated for Hardy-Weinberg equilibrium (HWE) and chi-square was calculated. If the obtained chi-square was smaller than the number in the statistics table (3.8), then the group is under HWE. Appropriate genetic models were selected to analyze the data according to the HWE in case and control groups. If the control group is under HWE but the case group is not, a recessive/dominant genetic model is appropriate for statistical analysis. If both the case and control groups are in HWE, the multiplicative pattern is used. This method is based on alleles, not genotypes, and compares the total number of wild and mutant alleles in cases and controls regardless of genotypes. Another possible genetic model is the additive model which was 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. This model shows a clear trend of an increased number of heterozygous and mutant homozygous genotypes, with the risk for heterozygous genotypes being approximately half of the one for mutant homozygous genotypes (33). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated via logistic regression using SPSS statistics v22 and $p<0.05$ were considered statistically significant.

Results

DNA concentration of samples was in the range of 77-247 *ng/µl*. Optical Density (OD) 260/280 ratios of extracted DNAs were in the range of 1.78-1.89.

The frequency of genotypes obtained between case and control individuals and the statistical analysis results are summarized in tables 1 and 2. For MTHFR rs1801131 (T>G), neither case nor control group was in Hardy Weinberg equilibrium (chi^2=20.0946, chi^2=39.947, respectively) and the statistical analysis using the additive model showed no significant association between this SNP and male infertility (p=0.111). Regarding MTHFR rs2274976, control group was not in Hardy Weinberg equilibrium (chi^2=18.434) but the case group was in Hardy Weinberg equilibrium (chi^2=0.533). There was a significant association between the mutant homozygous form of this SNP and male infertility (OR: 10.00, CI: 3.203-31.225). Regarding FASLG rs80358238, both case and control groups were in Hardy Weinberg equilibrium (chi^2= 0.4777 and chi^2=0.0015, respectively). Statistical analysis using a multiplicative model showed no significant association between this SNP and male infertility (p=0.161). Considering FASLG rs12079514, neither case nor control group was in Hardy Weinberg equilibrium (chi^2= 32.195 and chi^2=56.060, respectively). Statistical studies showed a significant association between the heterozygous form of this SNP and male infertility. Neither case nor control group was in Hardy Weinberg equilibrium (chi^2=81.698 and chi^2= 76.768) with regard to GSTM1 rs1192077068. There was no association between this SNP and

Table 1. Distribution of MTHFR, FASLG, BRCA2, and GSTM1 genotypes in infertile patients and controls

	Cases (n=120) (No.) (%)	Controls (n=120) (No.) (%)	p *
Genotype			
MTHFR1801131TT	48 (39.3)	62 (51.7)	
MTHFR1801131GT	36 (29.5)	24 (20.0)	0.111
MTHFR1801131GG	38 (31.1)	34 (39.3)	
Genotype			
MTHFR2274976 GG	32 (26.7)	40 (33.3)	
MTHFR2274976 GA	56 (46.7)	76 (63.3)	<0.001
MTHFR2274976 AA	32 (26.7)	4 (3.3)	
Genotype			
FASLG 80358238 AA	84 (70.0)	72 (60.0)	
FASLG 80358238 AG	34 (28.3)	42 (35.0)	0.161
FASLG 80358238 GG	2 (1.7)	6 (5.0)	
Genotype			
FASLG 12079514 AA	33 (27.5)	16 (13.3)	
FASLG 12079514 AC	85 (70.8)	100 (83.3)	0.014
FASLG 12079514 CC	2 (1.7)	4 (3.3)	
Genotype			
GSTM1 1192077068 CC	95 (81.9)	107 (89.2)	
GSTM1 1192077068 CA	5 (4.3)	4 (3.3)	0.272
GSTM1 1192077068 AA	16 (13.8)	9 (7.5)	
Genotype			
BRCA2 11571833 AA	24 (20.0)	55(45.8)	
BRCA2 11571833 AT	28 (23.3)	40(33.3)	<0.001
BRCA2 11571833 TT	68 (56.7)	25(20.8)	
Genotype			
BRCA2 4987117 CC	102 (85.0)	110 (91.7)	
BRCA2 4987117 CT	13 (10.8)	6 (5.0)	0.221
BRCA2 4987117 TT	5 (4.2)	4 (3.3)	

* p-values were obtained from χ^2 test

Table 2. Odds ratios for male infertility across different genotypes of MTHFR, FASLG, BRCA2, and GSTM1

	(OR; 95%CI)	p
Genotype (additive model)		
MTHFR1801131TT	1.00	
MTHFR1801131GT/TT	1.938 (1.022-3.672)	0.041
MTHFR1801131GG/TT	1.444 (0.795-2.622)	0.227
Genotype (additive model)		
MTHFR2274976 GG	1.00	
MTHFR2274976 GA/GG	0.921 (0.516-1.643)	0.781
MTHFR2274976 AA/GG	10.00 (3.203-31.225)	<0.001
Genotype (multiplicative model)		
FASLG 80358238 A	1.00	
FASLG 80358238 G	0.648 (0.409-1.027)	0.064
Genotype (additive model)		
FASLG 12079514 AA	1.00	
FASLG 12079514 AC/AA	0.412 (0.212-0.800)	0.008
FASLG 12079514 CC/AA	0.242 (0.040-1.466)	0.175
Genotype (additive model)		
GSTM1 1192077068 CC	1.00	
GSTM1 1192077068 CA/CC	1.408 (0.367-5.396)	0.738
GSTM1 1192077068 AA/CC	2.002 (0.846-4.742)	0.109
Genotype (additive model)		
BRCA2 11571833 AA	1.00	
BRCA2 11571833 AT/AA	1.604 (0.812-3.168)	0.226
BRCA2 11571833 TT/AA	6.233 (3.211-12.101)	<0.001
Genotype (additive model)		
BRCA2 4987117 CC	1.00	
BRCA2 4987117 CT/CC	2.337 (0.856-6.378)	0.090
BRCA2 4987117 TT/CC	1.348 (0.352-5.159)	0.742

male infertility (p=0.272). Moreover, both case and control groups were not in Hardy Weinberg equilibrium (chi^2=25.485 & chi^2=10.0148, respectively) regarding BRCA2 rs11571833. Obtained results showed a significant relation between the homozygous form of this SNP and male infertility. Finally, with respect to BRCA2 rs4987117, case or control groups were not in Hardy Weinberg equilibrium (chi^2=16.8637 and chi^2=35.627). No association was found between this SNP and male infertility (p=0.221).

Discussion

Sperm may not be able to fertilize the egg if it is immobilized, abnormal in shape, or the sperm count is low. Azoospermia (Absence of sperm in the semen) and severe oligospermia (Presence of fewer than 5 million *sperm/ml* of ejaculate) are very common among infertile men. Although some genetic abnormalities including structural and numerical chromosome abnormalities, deletion of the azoospermia factor region (AZF), and mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) have been identified as the causes of azoospermia and oligospermia in men, at least 40% of them still have no clear causes and are grouped as idiopathic infertility (34).

Problems with sperm production or quality may occur due to hormonal imbalance, damage to the urinary or genital organs, fever or heat stroke, some drugs and toxins, or some genetic disorders. Among genetic factors, polymorphisms in genes involved in general cellular functions such as oxi-

dation protection, cell death, DNA repair, etc. may influence the proper function of various cellular pathways including the spermatogenesis pathway (35). Therefore, these genes have gained special attention as they have key roles in development of many diseases. Few studies have been done on the association between polymorphisms of these genes with male infertility; yet, in most cases, the obtained results were conflicting. MTHFR is involved in homocysteine metabolism and its polymorphisms play role in DNA synthesis, methylation, and damage. Studies have shown that hypomethylation decreases genome stability and, consequently, during meiosis can lead to changes in methylation patterns which cause epigenetic defects (17, 36). It is reported that high levels of homocysteine lead to the production of hydrogen peroxide (H_2O_2) which is a harmful reactive oxygen and can cause DNA damage. On the other hand, oxidative stress can damage the plasma membrane of sperm and cause mitochondrial or genomic DNA defect (37). In this study, two SNPs of this gene were evaluated and no association between MTHFR rs1801131 (1298 A>C) and male infertility was found. Our findings indicated a significant relation between the MTHFR rs2274976 AA variant and idiopathic azoospermia. According to previous reports and meta-analyses regarding the study of two common SNPs of MTHFR gene, the results indicated that there was a significant association between MTHFR c.677 C>T variant and male infertility in azoospermia cases in the Asian population but no association was reported in 1298 A>C variant (8) which confirms our findings. Also, a relation between MTHFR rs2274976 G>A and nonsyndromic cleft lip (38, 39) and ischemic stroke was found in the Chinese population (40). There was no data about MTHFR rs2274976 G>A variant and male infertility to confirm or reject our findings.

GSTM1 is a polymorphic gene, and one of the eight classes of GSTs superfamily in which the main function is detoxifying free radicals, active electrophiles, environmental pollutants, and preventing DNA damage through lipid peroxidase formation (15). In the present study, the association of a stop-gained variant of GSTM1 rs1192077068 (G>A) with male infertility was investigated; however, the results showed no significant link between this variant and male infertility. This gene has a very common variant with the frequency of 23% to 63% among different populations (41). This deletion in homozygous forms lacks enzymatic activity in the cytosolic region of GST-M. Previous studies have suggested a link between this variant and infertility in men (1); however, no studies have re-ported the association of other polymorphisms of this gene with male infertility.

Mitochondrion is an important intracellular organelle that plays a key role in the production of ATP, ROSs, and triggering apoptosis; therefore, damage to mitochondria results in physiological disorders such as infertility (42). Increased frequency of single-strand or fragmented double-strand DNA caused by ROSs can induce apoptosis (43). Apoptosis is frequent in the spermatogenesis process. Approximately 75% of spermatogonial cells die before they reach puberty via apoptosis (24, 44). One of the pathways well known in the control of the germ cell population is the FAS/FASLG pathway in which FAS ligand binding to its receptor triggers apoptosis (23). A missense polymorphism of FASLG rs80358238 A>G was investigated in this study which is reported pathogenic in NCBI. Obtained results showed no significant correlation between this SNP and male infertility.

FASLG rs12079514 A>C was another missense SNP investigated in this study. There was a significant association between this SNP and male infertility in heterozygous form, which can be a good candidate for male infertility screening in cases with idiopathic azoospermia.

It seems that some physiological conditions, such as idiopathic male infertility, dramatically increase apoptosis (43). The role of FAS/FASLG polymorphisms in certain cancers has been identified (45, 46). Recently, there have been some reports of an association between FAS and FASLG polymorphisms with idiopathic male infertility. Regarding FAS and FASLG, genotype frequency is different among populations based on the ethnic differences in the populations; contradictory reports on the Chinese population can be regarded as a proof for this claim. Ju G et al. have reported an association between FASLG rs763110 C>T and idiopathic male infertility among Chinese (28) while others found no link between them (22). Reports indicate no association between this polymorphism and idiopathic infertility among the Iranian (47) and Indian (22) populations too.

The role of the BRCA2 gene in repairing double-strand DNA breaks and its mutations in the development of breast and ovarian cancer is known

(30). It is also reported that BRCA2 deficiency may lead to meiotic impairment and infertility (48). Obtained results showed a significant association between rs11571833 TT variant and male infertility, which introduce it as a good candidate for screening male infertility in cases with idiopathic azoospermia. But there was no significant difference between case and control groups in either heterozygous or homozygous cases between rs4987117 C>T variant of this gene and male infertility (p=0.221). Concerning the association of this gene with fertility, a cohort study by Kwiatkowski et al. showed that women carrying the mutation in the BRCA2 gene had better oocyte quality and fewer abortions (49). BRCA2 gene is expressed during spermatogenesis and has been suggested to be involved in meiotic recombination as it is localized on meiotic chromosome axis at synaptonemal complex stage (30, 50). A study in China showed the association between the N372H variant in the BRCA2 gene with male infertility in cases with azoospermia or severe oligospermia suggesting that polymorphisms of this gene may also play a role in male fertility (30). In our study, the effects of two variants of this gene (rs11571833 A>T, which produced a stop codon, and the rs4987117 C>T which is a missense variant) on male infertility in cases with idiopathic azoospermia or severe oligospermia were investigated. Obtained results showed a significant association between rs11571833 TT variant and male infertility, introducing it as a good candidate for screening male infertility in idiopathic cases. But there was no significant difference between case and control groups in either heterozygous or homozygous cases between rs4987117 C>T variant of this gene and male infertility (p=0.221). Although there have been no reports of a link between these SNPs and male infertility, previous studies have shown an increased risk of lung and breast cancer (51, 52) suggestive of possible effect of SNPs on other disorders.

This project was the first study that examined the association between novel variants of GSTM1, FASLG, MTHFR, and BRCA2 genes and prevalence of infertility among Iranian infertile men referred to Valiasr Tabriz. However, some limitations should be considered in the interpretation of findings. Some confounders including environmental factors, smoking, high risk jobs, *etc*. were not taken into account in this study. Therefore, the generalizability of the findings should be done cautiously.

Conclusion

In summary, this study provided evidence for the association of MTHFR rs2274976 AA variant, FASLG rs12079514 AC variant, and BRCA2 rs11571833 TT variant with male infertility, and suggested these polymorphisms as predisposing risk factors of male infertility in cases with idiopathic azoospermia. However, further studies with different ethnic populations and larger samples are needed to validate the findings. Investigation on the effect of these SNPs on gene expression is also suggested for future studies.

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Conflict of Interest

The authors declare that they have no competing interests.

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