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Original Article

Association of *MTHFR*, *NFKB1*, *NFKBIA*, *DAZL* and *CYP1A1* gene polymorphisms with risk of idiopathic male infertility in a Han Chinese population

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Abstract: In this study, we investigated the association between six genetic polymorphisms (*MTHFR* C677T and A1298C, *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G, *DAZL* A386G (T54A) and *CYP1A1* T3801C) and the risk of idiopathic male infertility in a Chinese population. A case-control study comprising 1,759 idiopathic male infertile patients of Han Chinese ethnicity and 1,826 healthy fertile control individuals was carried out. Genotypes of all polymorphisms were determined via PCR-RFLP. Chi-squared test and logistic regression modeling were performed to identify the association of the polymorphisms with idiopathic male infertility. It was found that the heterozygous and variant genotypes of the following polymorphisms were significantly associated with an increased idiopathic male infertility risk: *MTHFR* C677T (heterozygous OR=1.266 [1.089, 1.470], P=0.002; variant OR=1.384 [1.138, 1.684], P=0.001), *MTHFR* A1298C (heterozygous OR=1.233 [1.071, 1.419], P=0.004; variant OR=1.564 [1.183, 2.068], P=0.002), and *CYP1A1* T3801C (heterozygous OR=1.163 [1.007, 1.344], P=0.040; variant OR=1.232 [1.005, 1.510], P=0.045). When genotypes of non-significant polymorphisms were combined and analyzed, it was found that the combination between variant DD genotype of *NFKB1* -94ins/del ATTG polymorphism and heterozygous AG genotype of *DAZL* A386G polymorphism was significantly associated with a reduced idiopathic male infertility risk (OR=0.588 [0.376, 0.919], P=0.02). In summary, we have successfully identified the association (or lack thereof) between the polymorphisms and idiopathic male infertility risk.

Keywords: Association, idiopathic male infertility, oligoasthenoteratozoospermia, polymorphisms

Introduction

Infertility affects as much as 16.7% of all couples [1]. Approximately half of these infertility cases can be attributed to male factors [2]. A number of factors have been known to contribute to male infertility, including hypogonadism, genital infections, testicular maldescent, varicoceles, or exposure to environmental xenobiotics, among others [3]. However, in a large proportion (30-50%) of male infertility cases, no known cause has been pinpointed and the etiology remains poorly understood. These cases of male infertility are collectively known as idiopathic male infertility [4].

In recent years, a number of genetic abnormalities, including mutations, translocations and microdeletions, have been observed in cases of oligozoospermia and azoospemia [5]. In addition, several genetic factors have also been firmly demonstrated to cause failure in spermatogenesis [6]. From these observations, it could be postulated that genetic factors may also contribute, at least partially, to the etiology of idiopathic male infertility. A large number of genes are known to play a role in male gametogenesis, testicular development and metabolism of infertility-related xenobiotics. For example, *MTHFR* encodes the methylenetetrahydrofolate reductase enzyme, which is involved

Table 1. Details of PCR and restriction enzyme digestion

Polymorphism	Primers	Annealing temperature (°C)	Restriction enzyme	Genotype and band sizes
MTHFR C677T	Forward: 5'-TGA AGG AGA ACG TGT CTG CGG GA-3' Reverse: 5'-AGG ACG GTG CGG TGA GAG TG-3'	59	HinfI	TT (175, 23 bp) TC (198, 175, 23 bp) CC (198 bp)
MTHFR A1298C	Forward: 5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3' Reverse: 5'-CAC TTT GTG ACC ATT CCG GTT TG-3'	62	MboII	AA (56, 31, 30, 28, 18 bp) AC (84, 56, 31, 30, 18 bp) CC (84, 31, 30, 18 bp)
NFKB1 -94ins/del ATTG	Forward: 5'-TGG GCA CAA GTC GTT TAT GA-3' Reverse: 5'-CTG GAG CCG GTA GGG AAG-3'	60	Van91I	II (240, 45 bp) ID (281, 240, 45 bp) DD (281 bp)
NFKBIA 3'UTR A>G	Forward: 5'-GGC TGA AAG AAC ATG GAC-3' Reverse: 5'-GTA CAC CAT TTA CAG GAA GGG-3'	58	HaeIII	AA (424 bp) AG (424, 316, 108 bp) GG (316, 108 bp)
DAZL A386G (T54A)	Forward: 5'-GAA TGC TGA ATT TTT ACT CTT GAA G-3' Reverse: 5'-CTC TAT ACG TGG CTA GAG TTC-3'	62	AluI	TT (115, 66 bp) TA (115, 66, 53 and 13 bp) AA (115, 53 and 13 bp)
CYP1A1 T3801C	Forward: 5'-TAG GAG TCT TGT CTC ATG CCT-3' Reverse: 5'-AGC GGC TAC ACC TCT TCA CTG 3'	58	MspI	TT (340 bp) TC (340, 200, 140 bp) CC (200, 140 bp)

in folate metabolism (and hence, methylation) that is critical for spermatogenesis [7]. Apart from that, *NFKB1* encodes the NF-κB protein which regulates male germ cell apoptosis and gene expression during the process of spermatogenesis, while *NFKBIA* encodes a key inhibitor of NF-κB [8]. Besides, *DAZL* is an autosomal homologue of the *DAZ* gene cluster that is commonly deleted in azoospermic males [9]. In addition, it is thought that xenobiotic exposure may have a negative impact on male fertility, and *CYP1A1* encodes a phase I xenobiotic-metabolizing enzyme which detoxifies substances that could cause reproductive toxicity in men [10]. Proper regulation and functioning of these genes are critically important for optimal male fertility.

Polymorphisms in the above genes could alter the transcriptional activities of the genes (for *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G and *CYP1A1* T3801C polymorphism) or functions of the encoded proteins (for *MTHFR* C677T, *MTHFR* A1298C and *DAZL* A386G (T54A) polymorphisms). Thus, we hypothesize that polymorphisms in these genes could serve as risk factors for idiopathic male infertility. Several previous studies have addressed this hypothesis by investigating the association between the above polymorphisms and risk of idiopathic male infertility [8, 10-16], but inconclusive findings have been obtained in these reports. For example, while Naqvi et al. [11] showed a significant association between *MTHFR* C677T and an increased risk of idiopathic male infertility, Eloualid et al. [13] demonstrated an absence of significant association of the same polymorphism. Besides, while Teng et al. [15] demonstrated the important role of *DAZL* A386G (T54A) polymorphism in influencing idiopathic male infertility risk, Kumar et al. [16] showed no significant association between the polymorphism and the disease risk. A similar inconsistency in study findings can be observed for other polymorphisms. These inconclusive findings could be partially due to the insufficient study power attributed to the small sample size. In this study, we performed genetic association analysis on a large sample size in a Chinese population, with special focus on *MTHFR* C677T, *MTHFR* A1298C, *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G, *DAZL* A386G (T54A) and *CYP1A1* T3801C polymorphisms.

Materials and methods

Patients and controls

The study was done in accordance with the Declaration of Helsinki and approved by the institutional review board of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Informed consent was obtained from all subjects prior to their participation in the study. Two groups of study subjects were recruited: cases and controls. Cases comprised 1,759 idiopathic male infertile patients affected by idiopathic oligoasthenoteratozoospermia as defined by the World Health

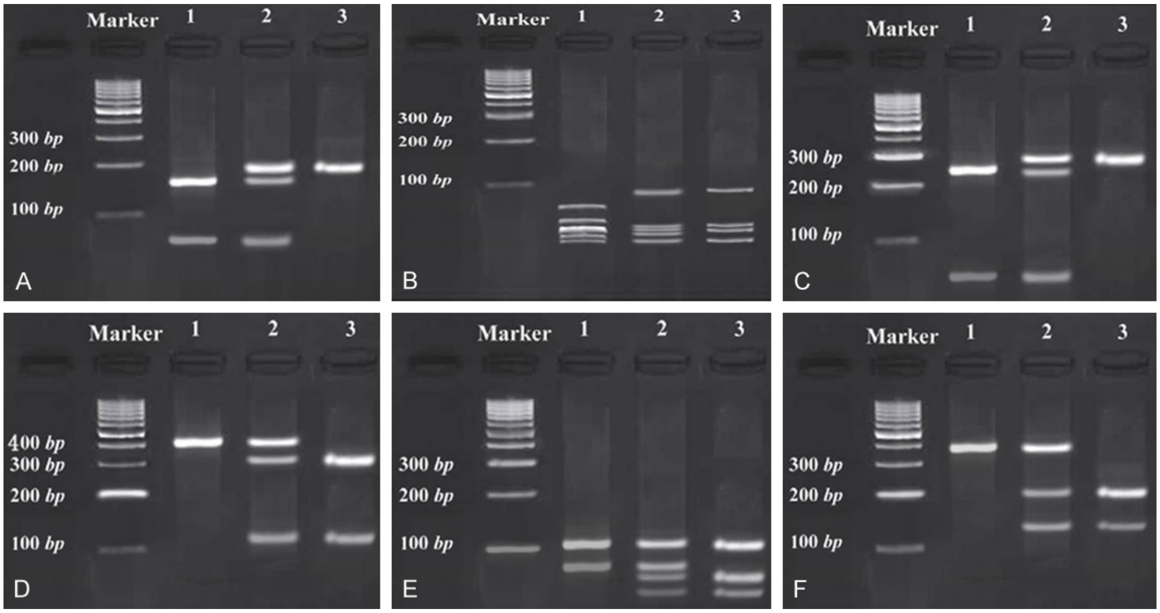

Figure 1. Band sizes for determination of the polymorphisms. (A) *MTHFR* C677T. Lane 1: TT (175, 23 bp), Lane 2: TC (198, 175, 23 bp), Lane 3: CC (198 bp) (B) *MTHFR* A1298C. Lane 1: AA (56, 31, 30, 28, 18 bp), Lane 2: AC (84, 56, 31, 30, 18 bp), Lane 3: CC (84, 31, 30, 18 bp) (C) *NFKB1* -94ins/del ATTG. Lane 1: II (240, 45 bp), Lane 2: ID (281, 240, 45 bp), Lane 3: DD (281 bp) (D) *NFKBIA* 3'UTR A>G. Lane 1: AA (424 bp), Lane 2: AG (424, 316, 108 bp), Lane 3: GG (316, 108 bp) (E) *DAZL* A386G (T54A). Lane 1: TT (115, 66 bp), Lane 2: TA (115, 66, 53 and 13 bp), Lane 3: AA (115, 53 and 13 bp) (F) *CYP1A1* T3801C. Lane 1: TT (340 bp), Lane 2: TC (340, 200, 140 bp), Lane 3: CC (200, 140 bp). All markers used were 100 bp ladder.

Organization criteria [17]. They were identified from patients who consecutively sought infertility treatment at Union Hospital for inability to conceive for at least 2 years of unprotected sex. Controls comprised 1,826 population-based individuals who had recently (within 3 months) fathered at least one child without the use of assisted reproductive technologies and showed normal semen parameters as defined by the World Health Organization criteria [17]. All subjects were of Han Chinese ethnicity and below 45 years of age. Subjects with urologic malignancies or other forms of sexual disorder, such as erectile dysfunction, were excluded from the study. Information on body mass index (BMI), smoking status and alcohol drinking habit of the study subjects were collected either from the medical records (for patients) or from face-to-face interview (controls).

Genotyping of polymorphisms

Genotyping of all the polymorphisms were performed via PCR-RFLP method. Genomic DNA was isolated from peripheral blood samples of the subjects using GeneAllExgene Blood SV Mini Kit (GeneAll Biotechnology Co, Ltd, Seoul, Korea) before subjected to PCR amplification using Promega PCR Master Mix (Promega Corporation, Madison, WI). The primers used for the PCR reaction and their corresponding annealing temperatures are shown in **Table 1**. PCR products generated from each reaction were digested with their respective restriction enzyme (also shown in **Table 1**). All digestion reactions were performed at 37°C overnight. The digested PCR products were separated on agarose gels and visualized under UV illuminator. Genotypes of the polymorphisms were determined based on the band sizes obtained, as shown in **Table 1** and **Figure 1**. The genotypes were validated via sequencing reactions using the same both forward and reverse primers as PCR.

Statistical analysis

All statistical analyses were performed using SPSS Version 20 (SPSS Inc., Chicago, IL). For demographic and clinical data, differences in categorical variables between cases and controls were evaluated using Pearson's chi-squared test, while differences of continuous variables were determined with Student's t

Table 2. Characteristics of cases and controls

Characteristic	Case	Control	P
Age	22-44 years	19-44 years	<0.001
Mean	32.91	31.74	
Standard deviation	6.78	7.50	
Body mass index			<0.001
Mean	23.54	23.03	
Standard deviation	2.18	2.16	
Smoking status			0.502
Never	825 (46.9%)	990 (54.2%)	
Ever	934 (53.1%)	836 (45.8%)	
Alcohol drinking habit			0.949
Never or occasional	815 (46.3%)	848 (46.4%)	
Regular*	944 (53.7%)	978 (53.6%)	

*Regular is defined as consuming at least 3 servings of alcohol per week.

test. Genotype distribution was tested for deviation from the Hardy-Weinberg equilibrium using chi-squared test. Difference in genotype distribution between cases and controls were also evaluated via chi-squared test. A logistic regression model was used to determine the association between the polymorphisms and risk of idiopathic male infertility, using wild type genotypes as the reference groups. P<0.05 was considered statistically significant.

Results

Overall, 1,759 idiopathic male infertile patients and 1,826 population-based fertile controls were recruited. The characteristics of the subjects are shown in **Table 2**. We recruited subjects below 45 years of age only, to prevent confounding due to decreased sperm quality associated with age. The ages of cases ranged from 22-44 years old, whereas the ages of the controls ranged from 19-44 years, with a mean 32.91 and 31.74 years respectively. Although the mean age did not differ much, the age difference between cases and controls was statistically significant (P<0.001). Similarly, statistical significance was observed for body mass index (BMI) of the subjects, with the mean BMI of cases being significantly higher (P<0.001) than that of controls (23.54 vs. 23.03). Majority of the subjects (53.1% cases and 54.2% controls) were ever smokers. Similarly, majority of the subjects (53.7% cases and 53.6% controls) were regular consumers of alcohol. No statistical significance was observed between cases and controls in terms of smoking status (P=0.502) and alcohol drinking habit (P=0.949).

We next investigated the genotype distribution of the six genetic polymorphisms (*MTHFR* C677T, *MTHFR* A1298C, *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G, *DAZL* A386G (T54A) and *CYP1A1* T3801C) among the cases and controls. The results are shown in **Table 3**. Among the controls, the distribution of all the six polymorphisms did not deviate significantly from the Hardy-Weinberg equilibrium (P>0.05). Statistically significant differences between cases and controls were observed for *MTHFR* C677T (P=0.001), *MTHFR* A1298C (P=0.001) and *CYP1A1* T3801C (P=0.035) polymorphisms, but not for *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G and *DAZL* A386G (T54A) polymorphisms (P>0.05). Thus, *MTHFR* C677T, *MTHFR* A1298C and *CYP1A1* T3801C polymorphisms were significantly associated with risk of idiopathic male infertility.

Next, we measured the magnitude of association between the three significant polymorphisms and risk of idiopathic male infertility, with the respective wild type genotypes served as the referent. The results are shown in **Table 3**. The heterozygous and variant genotypes of the three polymorphisms were found to be significantly associated with an increased risk of idiopathic male infertility. Specifically, for *MTHFR* C677T polymorphism, it was found that the CT heterozygotes showed a 1.266-fold risk increment of idiopathic male infertility compared to the referent CC genotype (P=0.002), while TT genotype showed a 1.384-fold risk increment (P=0.001). Besides, the AC genotype of *MTHFR* A1298C polymorphism showed an odds ratio of 1.233 (P=0.004), while the CC genotype showed an odds ratio of 1.564 (P=0.002). For *CYP1A1* T3801C polymorphism, the heterozygotes TC genotype showed a 1.163-fold increased risk of idiopathic male infertility (P=0.040), while the CC variant genotype was associated with a 1.232-fold risk increment at borderline significance (P=0.045), compared to the referent genotype.

Further, for the other three polymorphisms which did not show significant association

Table 3. Genotype distribution, conformation to HWE and odds ratio calculation

SNP and genotype	Case, N (%)	Control, N (%)	P (distribution)	P (control HWE)	Adjusted OR, 95% CI*	P (OR)
MTHFR C677T			0.001	0.805		
CC	513 (29.16)	638 (34.94)			Referent	
CT	907 (51.56)	887 (48.58)			1.266 (1.089, 1.470)	0.002
TT	339 (19.27)	301 (16.48)			1.384 (1.138, 1.684)	0.001
MTHFR A1298C			0.001	0.631		
AA	957 (54.41)	1097 (60.08)			Referent	
AC	670 (38.09)	632 (34.61)			1.233 (1.071, 1.419)	0.004
CC	132 (7.50)	97 (5.31)			1.564 (1.183, 2.068)	0.002
NFKB1 -94ins/del ATTG			0.656	0.544		
II	499 (28.37)	493 (27.00)			Referent	
ID	872 (49.57)	924 (50.60)			0.930 (0.795, 1.088)	0.366
DD	388 (22.06)	409 (22.40)			0.926 (0.767, 1.118)	0.423
NFKBIA 3'UTR A>G			0.835	0.278		
AA	348 (19.78)	365 (19.99)			Referent	
AG	832 (47.30)	877 (48.03)			1.002 (0.840, 1.196)	0.983
GG	579 (32.92)	584 (31.98)			1.048 (0.868, 1.265)	0.627
DAZL A386G (T54A)			0.074	0.142		
AA	1607 (91.36)	1634 (89.49)			Referent	
AG	152 (8.64)	190 (10.41)			0.838 (0.668, 1.050)	0.125
GG	0 (0.00)	2 (0.11)			-	-
CYP1A1 T3801C			0.035	0.850		
TT	625 (35.53)	723 (39.59)			Referent	
TC	860 (48.89)	849 (46.50)			1.163 (1.007, 1.344)	0.040
CC	274 (15.58)	254 (13.91)			1.232 (1.005, 1.510)	0.045

*Adjusted for age, BMI, smoking status and alcohol drinking habit.

alone (*NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G and *DAZL* A386G (T54A) polymorphisms), we investigated whether their combinations can lead to significant association. The results are shown in **Table 4**. Out of 27 possible genotype combinations, only the combination between variant DD genotype of NFKB1 -94ins/del ATTG polymorphism and heterozygous AG genotype of DAZL A386G polymorphism showed statistically significant association. This combination resulted in a reduced risk of idiopathic male infertility, with an odds ratio of 0.588 (P=0.020).

Discussion

In this study, we investigated the association of six genetic polymorphisms with the risk of idiopathic male infertility. We included only subjects below the age of 45, and adjusted our findings for age, BMI, smoking status and alcohol drinking habit of the subjects, as these parameters have been well-established to influence male fertility [18-21]. We showed that *MTHFR* C677T, *MTHFR* A1298C and *CYP1A1* T3801C polymorphisms were significantly associated with risk of idiopathic male infertility, with the heterozygous and variant genotypes of these polymorphisms being significantly overrepresented in the cases.

MTHFR encodes the methylenetetrahydrofolate reductase (MTHFR) enzyme, which plays an important role in folate metabolism. Specifically, MTHFR catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as the methyl donor for homocysteine during methionine synthesis. The methionine synthesized is then activated to form S-adenosylmethionine, which in turn serves as a major methyl donor during the process of DNA methylation. Appropriate DNA methylation is critical for spermatogenesis [7], and aberrant patterns of DNA methylation have been observed in sperm cells and testicular biopsies of infertile patients [22, 23]. Given the importance of proper DNA methylation in male fertility, catalytic activity of MTHFR enzyme must be kept at an optimal level. However, the

Table 4. Combinations of polymorphisms

SNP1	SNP2	Case, N (%)	Control, N (%)	Adjusted OR, 95% CI*	P (OR)
NFKB1 -94ins/del ATTG	*NFKBIA* 3'UTR A>G				
II	AA	97 (5.51)	106 (5.81)	Referent	
II	AG	230 (13.08)	232 (12.71)	1.134 (0.812, 1.584)	0.460
II	GG	172 (9.78)	155 (8.49)	1.254 (0.880, 1.788)	0.211
ID	AA	174 (9.89)	182 (9.97)	1.086 (0.766, 1.540)	0.641
ID	AG	409 (23.25)	447 (24.48)	1.028 (0.754, 1.401)	0.861
ID	GG	289 (16.43)	295 (16.16)	1.101 (0.797, 1.521)	0.559
DD	AA	77 (4.38)	77 (4.22)	1.109 (0.726, 1.694)	0.632
DD	AG	193 (10.97)	198 (10.84)	1.081 (0.767, 1.523)	0.657
DD	GG	118 (6.71)	134 (7.34)	0.994 (0.683, 1.445)	0.973
NFKBIA 3'UTR A>G	*DAZL* A386G (T54A)				
AA	AA	323 (18.36)	322 (17.63)	Referent	
AA	AG	25 (1.42)	43 (2.35)	0.617 (0.366, 1.041)	0.071
AA	GG	0 (0.00)	0 (0.00)	-	-
AG	AA	762 (43.32)	785 (42.99)	0.980 (0.814, 1.180)	0.832
AG	AG	70 (3.98)	91 (4.98)	0.778 (0.548, 1.106)	0.162
AG	GG	0 (0.00)	1 (0.05)	-	-
GG	AA	522 (29.68)	527 (28.86)	0.996 (0.817, 1.215)	0.971
GG	AG	57 (3.24)	56 (3.07)	1.073 (0.716, 1.607)	0.732
GG	GG	0 (0.00)	1 (0.05)	-	-
NFKB1 -94ins/del ATTG	*DAZL* A386G (T54A)				
II	AA	457 (25.98)	455 (24.92)	Referent	
II	AG	42 (2.39)	47 (2.57)	0.877 (0.564, 1.363)	0.559
II	GG	0 (0.00)	1 (0.05)	-	-
ID	AA	796 (45.25)	838 (45.89)	0.921 (0.781, 1.085)	0.326
ID	AG	76 (4.32)	85 (4.65)	0.896 (0.638, 1.258)	0.526
ID	GG	0 (0.00)	1 (0.05)	-	-
DD	AA	354 (20.13)	351 (19.22)	0.966 (0.792, 1.179)	0.735
DD	AG	34 (1.93)	58 (3.18)	0.588 (0.376, 0.919)	0.020
DD	GG	0 (0.00)	0 (0.00)	-	-

*Adjusted for age, BMI, smoking status and alcohol drinking habit.

MTHFR C677T and A1298C polymorphisms has been shown to result in a reduced catalytic activity of the enzyme [24], as the former causes an Ala-to-Val substitution at amino acid 222 of the enzyme and the latter leads to a Glu-to-Ala substitution at amino acid position 429. This reduced catalytic activity could cause compromised spermatogenesis [25], which explains the association of the two polymorphisms with an increased risk of idiopathic male infertility in this study. The finding that the *MTHFR* C677T polymorphism was associated with an increased risk of idiopathic male infertility agreed with several previous studies [11, 26-30], although a number of other studies failed to find an association between the polymorphism and idiopathic male infertility risk [13, 31-33]. Similarly, association of the A1298C polymorphism with idiopathic male infertility also agreed with some studies [13, 30, 35] and disagreed with the others [27, 29, 35, 36]. The discrepancy is not unexpected, as genetic associations are often confounded by a number of factors such as environmental exposure of the population studied or the sample sizes employed. Among all the studies on the association between *MTHFR* polymorphisms and idiopathic male infertility, the present work employed the largest sample size which provided the highest study power.

Besides MTHFR, cytochrome P450 1A1 (CYP1A1) also plays an important role in ensuring a non-compromised male infertility. There is growing evidence that exposure to environmental xenobiotics may lead to spermatogenetic failure [37, 38]. The CYP1A1 enzyme, which is vitally expressed in male reproductive organs, serves as a major metabolizer of environmental xenobiotics such as polycyclic aromatic hydrocarbons (PAHs) that can potentially lead to reproductive toxicity in men. This implicates a role of the CYP1A1 enzyme in idiopathic male infertility. It can be presumed that proper regulation of CYP1A1 level is essentially important for the optimal functioning of the enzyme. However, it is known that the *CYP1A1* T3801C polymorphism could influence its gene expression and mRNA stability [39], which explains the association of the polymorphism with risk of idiopathic male infertility which we observed in this study. Similar to our study, Vani et al. [40] observed a significant association between *CYP1A1* T3801C polymorphism and idiopathic male infertility. However, Salehi et al. [41] did not find such significant association. Intriguingly, Yarosh et al. [42] found a significant association only among smokers, but not among non-smokers. Similar to the *MTHFR* polymorphisms, the sample size used in this study was the largest compared to all other previous studies, which gives a higher statistical power to the present work.

In this work, we did not find any significant association of *NFKB1* -94ins/del ATTG, *NFKBIA* 3'UTR A>G and *DAZL* A386G (T54A) polymorphisms with risk of idiopathic male infertility. NF-κB, which is encoded by *NFKB1* gene, has been thought to regulate gene expression during spermatogenesis and induce male germ cell apoptosis during testicular stress [43-45]. In addition, the NF-κB pathway has been suggested as one of the pathways through which reactive oxygen species (ROS) causes reduced sperm quality [14]. Thus, NF-κB and its inhibitor, IκBα (which is encoded by the *NFKBIA* gene) definitely plays important roles in ensuring optimal male fertility, and polymorphisms of the two genes can potentially serve as risk factors for idiopathic male infertility. The *NFKB1* -94ins/del ATTG polymorphism contains two putative regulatory elements of the gene and has been shown to influence the mRNA expression of the gene. In contrast, the exact functional role of *NFKBIA* 3'UTR A>G polymorphism is not well understood, but since 3'UTR region plays essentially important roles in mRNA regulation [46], it is likely that nucleic acid substitution at this region may cause an altered gene expression. Despite this, the involvement of *NFKB1* and *NFKBIA* polymorphisms as risk factors for male infertility has not received much attention, with only one previous study investigated the former [8] and two previous studies investigated the latter [8, 14]. For *NFKB1* -94ins/del ATTG polymorphism, our observation of the lack of significant association with idiopathic male infertility did not agree with the previous work. However, for the *NFKBIA* 3'UTR A>G polymorphism, our finding concurred with Tek et al. [8] but disagreed with Yu et al. [14].

DAZL is an autosomal homolog of the DAZ gene cluster which is deleted in a proportion of azoospermic and oligozoospermic patients, which naturally warrants its investigation as a potential biomarker for idiopathic male infertility. *DAZL* is vitally expressed in male germ cells and encodes a RNA-binding protein that functions as a translational activator during spermatogenesis. The A386G polymorphism, which causes a Thr-to-Ala substitution at the 54^{th} amino acid (T54A substitution), occurs within a highly conserved RNA-recognition motif of its protein product and may cause disruptions to its biological activity. In one previous study in a Chinese population [15], the minor allele frequency of the *DAZL* A386G polymorphism was shown to be as high as 7.39%. However, this polymorphism was not detected in many other studies, suggesting its extremely low prevalence in the general population [46-49]. Using a large sample size, we attempted to provide a more accurate estimation of the minor allele frequency of this polymorphism in the Chinese population. Similar to other previous studies, we observed a low frequency of the variant allele in our population. However, in contrast to other previous studies [15, 50, 51], we observed a higher frequency of the variant allele in controls compared to the cases (controls: 5.32%, cases: 4.32%), although the difference was not statistically significant. We also observed that the combination between the variant allele of *DAZL* A386G polymorphism with the homozygous deletion genotype of the *NFKB1* polymorphism was significantly associated with a reduced risk of idiopathic male infertility. It is unknown whether this association arises as a result of interactions between the two polymorphisms, or arises spontaneously due to the small number of subjects carrying the combination genotypes (34/1.93% in cases; 58/3.18% in controls). However, given that no known interaction has been reported for the two genes and that statistically significant association was not observed for other genotype combinations, we propose that this finding could be a false-positive result.

In conclusion, we showed that *MTHFR* C677T, *MTHFR* A1298C and *CYP1A1* T3801C polymorphisms could serve as risk factors for idiopathic male infertility in the Chinese population. The strength of this present work is the large sample size used. However, we did not validate our finding in an independent set of study subjects and perform functional analysis of these polymorphisms. Future studies should address these issues to understand how exactly the polymorphisms can pose male infertility risk.

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Disclosure of conflictof interest

None.

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