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Association between methylenetetrahydrofolate reductase C677T polymorphisms and male oligozoospermia, asthenozoospermia or oligoasthenozoospermia: a case–control study

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Mutation of methylenetetrahydrofolate reductase (*MTHFR*) C677T leads to the decrease of folate utilization and the impairment of spermatogenesis. This study attempts to investigate the association between *MTHFR* C677T polymorphisms and nonobstructive oligozoospermia, asthenozoospermia or oligoasthenozoospermia in the Chinese population. The study cohort comprised 189 patients diagnosed with oligozoospermia, asthenozoospermia or oligoasthenozoospermia, and 626 controls based on clinical examinations. The *MTHFR* c.677 genotype of all subjects was determined by fluorescence staining in situ hybridization and the significance of different genotype frequencies was further analyzed by Chi-square test. The results showed that the frequency of *MTHFR* 677 CT genotype in the oligozoospermia, asthenozoospermia and oligoasthenozoospermia group was 33.3%, 38.3% and 44.0% respectively, whereas it was 47.3% in the control group. The *P* value of Chi-square test was 0.070, 0.103 and 0.654, respectively. The frequency of *MTHFR* 677 TT genotype in the oligozoospermia, asthenozoospermia and oligoasthenozoospermia group was 31.1%, 11.7% and 18.0% respectively, while that in the control group was 19.5%. The *P* value of Chi-square test was 0.061, 0.070 and 0.066, respectively. Collectively, there is a weak association between *MTHFR* C677T polymorphisms and oligozoospermia, asthenozoospermia or oligoasthenozoospermia within the current Chinese population cohort.

Keywords Folate, *MTHFR*, Oligozoospermia, Asthenozoospermia, Oligoasthenozoospermia

In recent years, the infertility rate has increased worldwide due to various harmful factors such as life pressure, environmental deterioration and food contamination. According to statistical data, the infertility rate among individuals of childbearing age ranges from 8 to 12%, with male subjects accounting for 50% of these cases[1]. Semen abnormalities caused by spermatogenic disorders are the most common cause of male infertility, although various etiologies have been found in recent years. The main manifestations of semen abnormalities encompass oligozoospermia or asthenozoospermia, thus improving these conditions is an effective strategy to treat male infertility[2,3].

The pathogenesis of oligozoospermia or asthenozoospermia is highly intricate. Over the past few decades, DNA methylation abnormalities and oxidative stress in spermatozoa have become a great challenge to male infertility. Folate, also known as vitamin B_9, is an indispensable nutrient that plays a critical role in various biological processes, including DNA synthesis and repair, RNA synthesis and cellular growth[4]. It also exhibits potent antioxidant properties, thereby protecting cells from oxidative stress caused by free radicals[5]. Clinical experiments have demonstrated that folate supplementation enhances sperm production and improves sperm

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Parameters	Control ($n=626$)	Patient		
		Oligozoospermia ($n=45$)	Asthenozoospermia ($n=94$)	Oligoasthenozoospermia ($n=50$)
Age (years)	30.55 ± 3.93	29.91 ± 4.25	31.96 ± 4.22*	30.76 ± 3.65
BMI (kg/m^2)	24.16 ± 2.77	24.51 ± 2.5	23.99 ± 2.48	23.89 ± 3.22
Sperm concentration (10^6/mL)	63.41 ± 34.3	12.64 ± 6.87*	55.14 ± 39.37*	6.16 ± 5.06*
Total sperm count (10^6)	222.77 ± 131.95	39.51 ± 18.65*	176.62 ± 141.69*	21.8 ± 20.61*
Progressive motility (%)	50.95 ± 10.35	47.8 ± 9.58*	21.44 ± 7.75*	9.82 ± 11.36*

Table 1. Demographic data on patients and basic semen analysis data for each group. Data are presented as mean ± SD. All parameters were compared using Mann-Whitney U Test. *Represents $P<0.05$ compared to control group.

SNP	Group	Genotype	Measured value	Theoretical value	*P* value	χ^2
MTHFR C677T (RS1801133)	Patient	CC	82 (43.4%)	74 (39.2%)	0.06	5.74
		CT	73 (38.6%)	89 (47.1%)		
		TT	34 (18.0%)	26 (13.8%)		
	Control	CC	208 (33.2%)	203 (32.4%)	0.66	0.82
		CT	296 (47.3%)	307 (49.0%)		
		TT	122 (19.5%)	116 (18.5%)		

Table 2. Hardy-Weinberg equilibrium of *MTHFR* C677T genotype in the patient and control group.

motility[6,7]. In fact, folate can not be synthesized internally in the human body, and it must be obtained through external intake. Folate is metabolized into 5,10-methylenetetrahydrofolate, which is subsequently reduced to 5-methyltetrahydrofolate by 5,10-methylenetetrahydrofolate reductase (MTHFR). 5-methyltetrahydrofolate and vitamin B_{12} are converted to methionine through the action of methionine synthetase. Methionine is further metabolized to form S-Adenosylmethionine, which is a methyl donor involved in the methylation process of DNA, RNA, amino acid and protein. Therefore, MTHFR plays a pivotal role in both the folate cycle and the homocysteine cycle as it regulates methyl donor availability while influencing homocysteine levels[8]. Polymorphisms were identified at the *MTHFR* gene loci, especially C677T (RS1801133), which have been associated with decreased enzyme activity, impaired folate utilization, and potential impact on spermatogenesis.

The present case-control study attempts to investigate the association between *MTHFR* C677T polymorphisms and oligozoospermia, asthenozoospermia or oligoasthenozoospermia in the Chinese population, with an aim to enhance understanding of their pathogenesis.

Results

The polymorphisms of *MTHFR* C677T are in Hard-Weinberg equilibrium

A total of 1049 men were tested for *MTHFR* genotype, including 13 individuals with BMI ≧ 30 kg/m^2, 10 individuals aged > 45, 169 individuals with genitourinary infection, 3 individuals with varicocele, and 39 individuals whose semen analysis was conducted after gene detection. Thus, a total of 234 men were excluded from the study, leaving a final sample size of 815 men included in the case-control study: 626 in the control group and 189 in the patient group. The patient group comprised of 45 cases with oligozoospermia, 94 cases with asthenozoospermia and 50 cases with oligoasthenozoospermia. The demographic characteristics and basic semen analysis data from four groups were summarized in Table 1. The sperm concentration, count or progressive motility (PR) were significantly decreased in the patient group compared to the healthy control group.

Subsequently, we conducted an analysis of *MTHFR* C677T polymorphisms in both the control group and the patient group. The distribution of genotypes is summarized in Table 2. The Chi-square test results indicate that the *P* value for the measured and theoretical values exceeds 0.05. Certainly, the results of the control group and the patient group are in Hardy-Weinberg equilibrium.

The association between *MTHFR* C677T polymorphisms and male oligozoospermia, asthenozoospermia or oligoasthenozoospermia in the current cohort is not significant

The distribution of *MTHFR* C677T polymorphisms in the control group and the oligozoospermia group was summarized in Table 3. In the control group, the frequencies of CC, CT, TT genotypes of *MTHFR* C677T polymorphisms were 33.2%, 47.3% and 19.5% respectively, while in the oligozoospermia group they were 35.6%, 33.3% and 31.1% respectively. The Chi-square test results showed that there was no significant difference ($P=0.070$) in the frequency of CT genotype between the control group and the oligozoospermia group, as well as for TT genotype ($P=0.061$).

SNP	Genotype	Control ($N=626$)	Oligozoospermia ($N=45$)	*P* value	OR (95% CI)
MTHFR C677T (RS1801133)	CC	208 (33.2%)	16 (35.6%)		
	CT	296 (47.3%)	15 (33.3%)	0.070	0.557 (0.294–1.056)
	TT	122 (19.5%)	14 (31.1%)	0.061	1.866 (0.963–3.615)

Table 3. Genotype distribution of *MTHFR* C677T in the control and oligozoospermia group.

SNP	Genotype	Control ($N=626$)	Asthenozoospermia ($N=94$)	*P* value	OR (95% CI)
MTHFR C677T (RS1801133)	CC	208 (33.2%)	47 (50.0%)		
	CT	296 (47.3%)	36 (38.3%)	0.103	0.692 (0.444–1.079)
	TT	122 (19.5%)	11 (11.7%)	0.070	0.548 (0.283–1.059)

Table 4. Genotype distribution of *MTHFR* C677T in the control and asthenozoospermia group.

SNP	Genotype	Control ($N=626$)	Oligoasthenozoospermia ($N=50$)	*P* value	OR (95% CI)
MTHFR C677T (RS1801133)	CC	208 (33.2%)	19 (38.0%)		
	CT	296 (47.3%)	22 (44.0%)	0.654	0.876 (0.490–1.565)
	TT	122 (19.5%)	9 (18.0%)	0.066	0.907 (0.429–1.916)

Table 5. Genotype distribution of *MTHFR* C677T in the control and oligoasthenozoospermia group.

Similarly, there was no statistically significance observed in the frequency of *MTHFR* C677T genotypes between the control group and the asthenozoospermia group or the oligoasthenozoospermia group (Tables 4 and 5).

Discussion

The MTHFR is a crucial regulator of folate metabolism, playing key roles in DNA methylation and spermatogenesis[9,10]. A common SNP of *MTHFR* gene, c.677 C>T(RS1801133), leads to decreased catalytic activity and increased heat tolerance of MTHFR. This SNP causes an alanine to valine substitution at position 222 (p. Ala222Val). Previous studies showed that the *MTHFR* C677T mutation reduces the enzyme activity of MTHFR by 35% in persons who are heterozygous and by 70% in homozygous population[11]. Generally, mutations in the *MTHFR* gene that decrease enzyme activity leads to the decrease of 5-methyltetrahydrofolate production, which may further impact sperm production and motility.

Increasing studies have been conducted on the association between *MTHFR* C677T polymorphisms and oligozoospermia or asthenozoospermia. The fifth edition of the WHO laboratory manual for the examination and processing of human semen was implemented in 2010[12]. Considering the disparities from previous iterations of implementation standards, we conducted an extensive literature search encompassing articles published after 2010 and found 4 pertinent studies. The results of the association study between *MTHFR* C677T polymorphism and male oligozoospermia or asthenozoospermia are inconsistent. Gurkan et al.. reported that the *MTHFR* 677 TT genotype was identified as a significant risk factor for male oligozoospermia in the Trakya region of Turkey[13]. However, Eloualid's team found no association between *MTHFR* 677 CT and TT genotypes and the risk of oligozoospermia in Casablanca, Morocco[14]. In a case-control study conducted by Xie et al.., it was demonstrated that the *MTHFR* 677 TT genotype posed a risk for both oligozoospermia and asthenozoospermia within the Chinese Han population. Additionally, they confirmed that folate supplementation could effectively increase sperm density among patients with the *MTHFR* 677 genotype mutation[7]. Inconsistently, Najafipour et al.. reported no significant association between the *MTHFR* 677 CT and TT genotypes and the risk of oligozoospermia or asthenozoospermia in Tehran, Iran[15]. Thus, the association between *MTHFR* C677T polymorphisms and male oligozoospermia or asthenozoospermia may be influenced by ethnicity background. In the present case-control study, we attempted to investigate the association between *MTHFR* C677T polymorphisms and oligozoospermia, asthenozoospermia or oligoasthenozoospermia. Contrary to the findings of Xie et al.., our results showed that there is no significant association among this Chinese population cohort. This inconsistent result may be attributed to the fact that the research subjects come from different regions in China. Another possible reason is that, with the rapid economic development of China, people may pay more attention to balanced diet and folate intake. Dietary folate supplements can improve the decrease of 5-methyltetrahydrofolate caused by decreased MTHFR activity.

Compared with previous studies, we have augmented the sample size and categorized patients into groups based on oligozoospermia, asthenozoospermia and oligoasthenozoospermia. The results are more accurate and reliable. However, there are certain limitations in the study: (1) The patient group was not further stratified based on disease severity. (2) This study did not further subdivide subjects from different geographical regions. In the future, we intend to categorize patients into subgroups based on both disease severity and region, and study their potential association with *MTHFR* C677T polymorphisms.

Conclusion

The *MTHFR* 677 genotypes in this case-control cohort showed a weak association with male oligozoospermia, asthenozoospermia or oligoasthenozoospermia within the Chinese population. It is still controversial to identify the etiology of oligozoospermia, asthenozoospermia or oligoasthenozoospermia through detecting *MTHFR* 677 genotypes.

Methods

Statement

The oral mucosal epithelial cells were processed following the genetic testing protocol established by the Molecular Laboratory of Hangzhou Women's Hospital. The study received approval from the Ethics Committee of Hangzhou Women's Hospital (NO.2022-4-06), and informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

Subjects

Patients who received comprehensive pre-childbearing examinations at Hangzhou Women's Hospital from December 1, 2021 to March 31, 2024 were included in the study. According to the patient's previous medical history, semen analysis, gene detection, ultrasound and other relevant imaging examination, patients with the following outcomes were excluded. Exclusion criteria encompass abnormal karyotype and Y chromosome abnormalities, varicocele, history of cryptorchidism, severe genitourinary system infection or surgery, semen analysis conducted after gene detection, history of obvious exposure to environmental pollutants, > 45 years old, utilization of cytotoxic drugs, immunosuppressants, antiviral drugs, androgen or anti-androgen agents, and a body mass index (BMI) ≥ 30 kg/m^2.

Control group ($n=626$): men who underwent semen analysis exhibited normal sperm motility and count. Patient group: men diagnosed with oligozoospermia ($n=45$), asthenozoospermia ($n=94$) or oligoasthenozoospermia ($n=50$). According to the diagnostic criteria outlined in the 5th edition of WHO laboratory manual for the examination and processing of human semen: asthenozoospermia is identified when progressive motility (PR) $<32\%$; oligozoospermia is defined as a total sperm count $<39\times10^6$ or sperm concentration $<15\times10^6$/ml; the simultaneous presence of both conditions falls under the category of oligoasthenozoospermia group.

Genotyping

The samples (Oral mucosal epithelial cells) from the participants were collected and used for DNA extraction. Briefly, the sampling swads were immersed and agitated in a 0.74% NH_4Cl extract for 20 s. Then, the samples were centrifuged at 3000 rpm for 1 min to obtain white precipitates. Cell preservation solution was thoroughly mixed with the precipitates to prepare for further testing. The prepared samples were added to the universal sequencing reaction kit (Jinan Guangyin Medical Science and Technology Co., Ltd) following the provided instructions. The genotype of each sample was determined through fluorescence staining in situ hybridization.

Statistical analyses

The demographic data on patients and basic semen analysis data were presented as mean ± SD, and all parameters were compared using Mann-Whitney U Test. The samples included in the study were subjected to a Hardy-Weinberg equilibrium test. Chi-square test was used to calculate the difference of genotype distribution between control and patient groups. An unconditional logistic regression method was used to calculate odds ratio (OR) and 95% confidence interval (CI). $P<0.05$ is statistically significant.

Data availability

The raw data is provided within supplementary material files. To ensure the privacy of the subjects, we have redacted any identifying information.

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Author contributions

Fu-jia Ren drafts the manuscript; Zhi-yong Zhang, Fu-jia Ren and Guo-ying Fang contribute to the conception and design of the study; Fu-jia Ren and Zhi-yong Zhang contribute to the acquisition, analysis, or interpretation of the data for the work; All authors approve the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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