



Research Article

MTHFR C677T Gene Polymorphism and Homocysteine in North China Patients with Varicocele

Yiping Xiu,¹ Zhiqiang Cao,² Dongyang Yu,^{1,3} Caleb Yang,⁴ Jiang Wang,⁵ Jiancheng Pan,⁵ Zhongcheng Xin ,⁵ and Yuhong Feng ⁵

¹Department of Urological Plastic Surgery, General Hospital of the Northern Theater Command, Shenyang 110004, China

²Department of Urology, Shengjing Hospital Affiliated with China Medical University, Shenyang 110004, China

³Graduate School of China Medical University, Shenyang 110004, China

⁴Department of Nutritional Sciences and Toxicology, University of California, Berkeley 94702, USA

⁵Male Reproductive and Sexual Medicine, Department of Urology, The Second Hospital of Tianjin Medical University, Tianjin 300202, China

Correspondence should be addressed to Zhongcheng Xin; xinzc@bjmu.edu.cn and Yuhong Feng; mnwkyh@163.com

Received 2 November 2022; Revised 7 November 2023; Accepted 19 March 2024; Published 9 April 2024

Academic Editor: Haney Samir

Copyright © 2024 Yiping Xiu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

To investigate the correlation between methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism, homocysteine, and male infertility with varicocele in North China. One Hundred infertile males with varicocele (VC; grade II-III, VC group) and 100 healthy males with normal semen parameters and no varicocele (NC group) were recruited for PCR microarray, blood and semen testing. Compared with the CC genotype in the NC group, the TT genotype in the NC group and the CC genotype in the VC group showed no significant changes in sperm motility ($P = 0.191$; $P = 0.130$), sperm density ($P = 0.591$, $P = 0.643$), plasma homocysteine level ($P = 0.511$; $P = 0.677$), and seminal plasma MDA ($P = 0.752$; $P = 0.451$). In contrast, VC patients with the TT genotype had higher plasma homocysteine level and seminal plasma MDA levels ($P < 0.001$), lower partial pressure of oxygen in seminal pulse (PO_2 ; $P < 0.001$) and poorer sperm quality ($P < 0.001$), as compared with the CC genotype. This suggests that MTHFR C677C>T may not be a risk factor for male Varicocele in North China. However, this may affect the oxidative stress associated with homocysteine expression, which in turn affects semen parameters in VC patients. Larger studies are needed to validate our findings.

1. Introduction

According to global statistics, one-quarter to one-fifth of couples will be affected by fertility problems. Among them, known male and female factors each account for 25%, and unexplained infertility accounts for 50% [1–3]. One-third of the known male infertility factors are related to varicocele (VC). VC is one of the most common diseases of adolescent and reproductive-age men and one of the main causes of male infertility during the reproductive period [4]. VC occurs from a combination of genetic susceptibility and acquired factors [5], but the exact mechanism is unclear [6, 7]. VC affects the pathogenesis of male testicular function and causes infertility, which is related to multiple factors, such as high temperature, hypoxia, and oxidative stress damage [5, 8, 9], of which

oxidative stress damage is the core cause of VC-associated infertility. Recent studies have found that homocysteine (Hcy) has many adverse effects such as inducing oxidative stress, promoting inflammation and damage to vascular endothelial cells, stimulating vascular smooth muscle cell proliferation, and promoting thrombosis [10, 11]. Therefore, Hcy was considered to be a potential factor in the occurrence of VC and in the reduction of sperm quality.

The 1-carbon unit cycle is an important amino acid synthesis pathway that includes two cycles of folic acid and methionine, as well as the transsulfuration pathway. During the methionine and folate cycles, Hcy is regulated [12]. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in the metabolism of folate and methionine and can catalyze the reduction of 5,10-methylenetetrahydrofolate to

5-methyltetrahydrofolate [13]. 5-Methyltetrahydrofolate is a substrate required for Hcy methylation catalyzed by methionine synthase. The final product is methionine, and then two main antioxidant molecules are synthesized: taurine and glutathione [14]. This process can maintain the normal level of plasma Hcy [12, 15]. When the 677th base cytosine (C) of the MTHFR gene is mutated to thymine (T), the MTHFR activity will decrease. MTHFR with a homozygous mutant (TT) at this site has the lowest enzyme activity, which leads to hyperhomocysteinemia (HHcy) [16, 17]. Several studies had shown that the MTHFR C677T gene polymorphism might have a specific correlation with the incidence VC and infertility [18–21]. But recent researches in different locations and ethnicities indicated contradictory conclusion [22, 23].

Due to the existence of the above controversy and the differences of gene mutation frequency in different populations and a lack of large-scale casecontrol studies in men in North China. This study aims to explore the relationships between the MTHFR C677T gene polymorphism and Hcy and male infertility with VC in men in North China.

2. Materials and Methods

2.1. Research Subjects. This case-control study recruited 100 male patients with primary infertility and grade II-III varicocele (VC group) from the department of urology, and 100 healthy young volunteers (18–30 years old) (normal control, NC group) at the physical examination centre, The Second Hospital of Tianjin Medical University, from December 2019 to October 2020. Experiments were approved by local ethics committee (NO.:2018-IRB-076), in compliance with national guidelines for the care and use of clinical samples.

The inclusion criteria were as follows: 18 years of age or older, and male infertility for more than 1 year, and second-grade or third-grade VC with positive reflux. The exclusion criteria were as follows: patients with grade I VC, smoking, excessive alcohol use, drug consumption, previous VC repair, the presence of other infertility-related diseases, patients with VC combined with haematological diseases, endocrine diseases, cancer or systemic infectious diseases, patients who had taken vitamin B or E or folic acid within 1 month, or other antioxidant treatments, and patients with VC combined with acute epididymitis or prostatitis.

This study was reviewed and approved by the local medical ethics committee. Only VC patients underwent the microsurgical ligation. VC patients and their families were informed of the possible consequences of performing microsurgical spermatic ligation. Both VC patients and healthy volunteers were required to provide data and samples and to sign an informed consent form.

2.2. Methods

2.2.1. Clinical Data Collection. Clinical data, including age, body mass index (BMI), and distribution of the MTHFR C677T gene and allele frequencies was collected from the two groups.

2.2.2. Semen Analysis. In the VC and NC groups, patients were asked to practice abstinence for 5–7 days. Semen

obtained by masturbation was analyzed using an automatic semen analyzer (BEION, Shanghai, China) for routine semen analysis and examination within half an hour, adopting the WHO (No. fifth edition) semen examination standard. Sperm density was recorded with normal reference $>2.0 \times 10^6 \text{ mL}^{-1}$; sperm motility was recorded with normal reference PR+NP $>40\%$.

2.2.3. Hcy Concentration Detection and Genotype Analysis. In the VC and NC groups, 2 mL of peripheral venous blood was drawn from each patient. A fully automatic biochemical analyzer (Beckman Coulter, USA) was used to determine the Hcy level using a cyclic enzymatic method. The normal reference range is 0–15 $\mu\text{mol L}^{-1}$.

2.2.4. Determination of Seminal Plasma Malondialdehyde (MDA). The semen samples collected following the masturbation test were frozen immediately after liquefaction. All samples were transferred to the laboratory while frozen and thawed within a week, and the seminal plasma MDA value was analyzed according to the instructions of the kit (Jiancheng, Nanjing).

2.2.5. Determination of the MTHFR Gene Polymorphism. A blood genome extraction kit was used to extract genomic DNA of peripheral venous blood. After PCR amplification, an MTHFR (C677T) gene detection kit (Sanji Bio, Changsha) was used for PCR amplification and sequencing. The patient was genotyped using a biochip reader.

2.2.6. Intraoperative Spermatic Venous Oxygen Saturation PO_2 of VC Patients. In the VC group, the spermatic vein was ligated under a microscope under general anesthesia, the arteries and lymph vessels were preserved, and the internal and external spermatic veins were ligated. After the spermatic cord was exposed, the internal spermatic vein was separated and punctured with a blood gas needle to collect blood from the internal spermatic cord for blood gas analysis.

2.3. Statistical Analysis. Statistical analysis was performed using IBM SPSS statistical software (version 22.0). Continuous data related to Hcy levels and sperm density, among others, were expressed as mean \pm standard deviation. χ^2 test was used to assess categorical data related to the distribution and frequency of genetic polymorphisms in order to analyze differences between groups. Independent samples *t*-test or ANOVA was used to compare measurements between groups. $P < 0.05$ indicated statistically significant differences.

3. Results

3.1. General Information. There were 100 cases in the VC group and 100 cases in the NC group. In the VC group, ages ranged from 18 to 37 years, and 75 cases of left-sided varicosities and 25 cases of bilateral varicosities, all of which were varicocele above grade I. In the NC group, ages ranged from 18 to 32 years old, and healthy volunteers with normal semen parameters were excluded from the diagnosis of VC by physical examination and color Doppler ultrasound in Standing position.

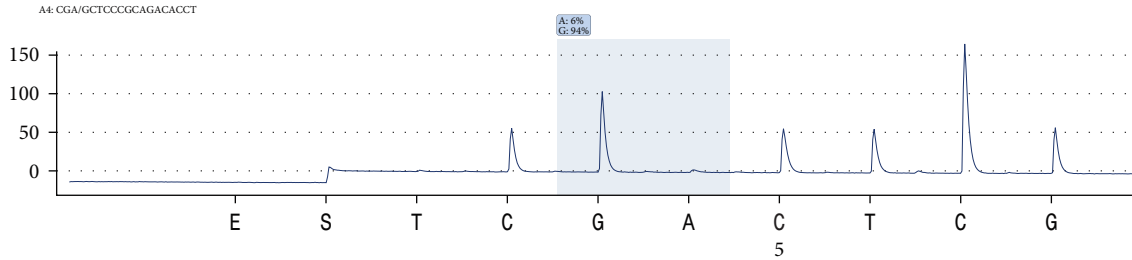
Related results peak chart:

MTHFR (C677T; retest)

Sequence of standard wild type target: GGGAGCCGATT (the red area is the wild-type sequence of the polymorphic site)

Sequence of mutant type: GGGAGTCGATT (red areas are polymorphic site mutant sequences)

Sequence of detection result: GGGAGCCG



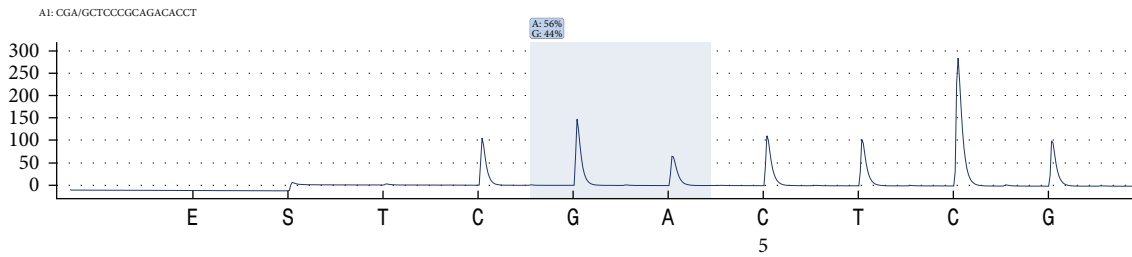
(a)

MTHFR (C677T; retest)

Sequence of standard wild type target: GGGAGCCGATT (the red area is the wild-type sequence of the polymorphic site)

Sequence of mutant type: GGGAGTCGATT (red areas are polymorphic site mutant sequences)

Sequence of detection result: GGGAGC/TCG



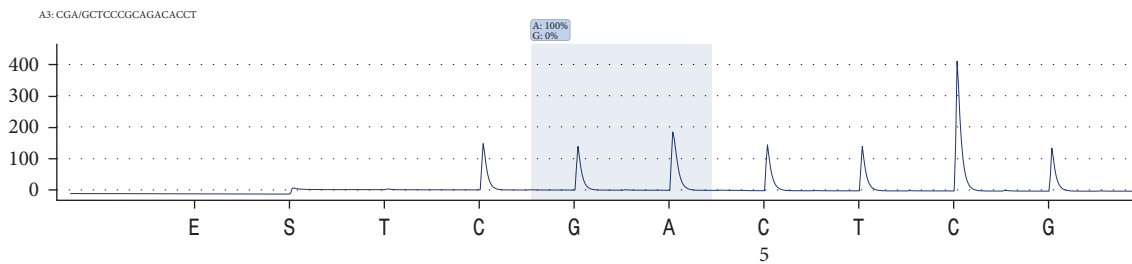
(b)

MTHFR (C677T; retest)

Sequence of standard wild type target: GGGAGCCGATT (the red area is the wild-type sequence of the polymorphic site)

Sequence of mutant type: GGGAGTCGATT (red areas are polymorphic site mutant sequences)

Sequence of detection result: GGGAGTCG



(c)

FIGURE 1: Gene polymorphism sequencing diagram: (a) CC, (b) CT, and (c) TT.

3.2. *MTHFR* C677T Gene Polymorphism Test Results. The distribution of *MTHFR* polymorphisms in each group was analyzed, including 100 VC patients and 100 healthy volunteers with normal semen parameters and no VC. Three genotypes—cytosine–cytosine (CC), cytosine–thymine (CT), and thymine–thymine (TT)—were determined (Figure 1). The

results showed that among the 100 patients in the VC group, 37 patients had the CC genotype, 48 patients had the CT genotype, and 15 patients had the TT genotype. The frequency of the T allele was 39%. Among the 100 subjects in the NC group, 35 subjects had the CC genotype, 49 subjects had the CT genotype, 16 subjects had the TT genotype, and the frequency

TABLE 1: Participant characteristics and distribution of the MTHFR C677T gene and allele frequencies of subjects (varicocele and control groups).

Genotype	Control		Varicoceles	
			II	III
CC	35		17 (45.9%)	20 (54.1%)
<i>P</i>	—			0.432
CT	49		21 (43.8%)	27 (56.2%)
<i>P</i>	—			0.367
TT	16		9 (60.0%)	6 (40%)
<i>P</i>	—			0.158

Note. Values are mean \pm standard deviation. Abbreviations: CC, cytosine–cytosine; CT, cytosine–thymine; TT, thymine–thymine; BMA, body mass index.

TABLE 2: Frequency distributions of the selected variables between the varicocele grade II and III.

Group (<i>n</i>)	Value age (y)	BMI (kg/m ²)	Genotype frequency (%)			Allele frequency (%)	
			CC	CT	TT	C	T
VC (100)	26.42 \pm 7.24	25.43 \pm 3.24	37 (37.0%)	48 (48.0%)	15 (15.0%)	122 (61.0%)	78 (39.0%)
NC (100)	27.73 \pm 6.45	26.57 \pm 5.73	35 (35.0%)*	49 (49.0%)*	16 (16.0%)*	119 (59.5%)*	81 (40.5%)*
<i>P</i>	0.354	0.124	0.435	0.376	0.576	0.368	0.437

Note. Values are mean \pm standard deviation. $\alpha = 0.05$. * indicates the comparison of the VC group and NC group. Abbreviations: CC, cytosine–cytosine; CT, cytosine–thymine; TT, thymine–thymine; BMI, body mass index.

TABLE 3: Comparison of semen parameters, plasma homocysteine levels, and venous blood gas analysis between different genotypes of MTHFR C677T in the VC group and NC group.

Group	Genotype	Number	Sperm motility (PR+NP) (%)	Sperm density $\times 10^6$ mL ⁻¹	MDA in seminal plasma (nmol·mL ⁻¹)	Hcy (mmol·L ⁻¹)	PO ₂ of spermatic vein (mmHg)
VC (100)	CC	37	41.60 \pm 12.15	35.30 \pm 18.60	1.13 \pm 0.46	8.35 \pm 3.17	61.00 \pm 3.50
	CT	48	24.50 \pm 7.45	28.20 \pm 6.90	1.43 \pm 0.78	14.79 \pm 7.36	43.50 \pm 3.50
	TT	15	17.40 \pm 6.67*	16.45 \pm 9.75*	3.77 \pm 1.47*	19.84 \pm 6.67*	32.00 \pm 3.50*
* <i>P</i>	—	—	<0.001	<0.001	<0.001	<0.001	<0.001
NC (100)	CC	35	45.30 \pm 11.57 [#]	37.55 \pm 16.60 [#]	1.04 \pm 0.67 [#]	8.65 \pm 4.65 [#]	—
	CT	49	49.40 \pm 1.47	37.45 \pm 16.65	1.21 \pm 0.89	9.51 \pm 5.77	—
	TT	16	41.34 \pm 6.67 [§]	39.80 \pm 14.45 [§]	1.12 \pm 0.54 [§]	9.68 \pm 4.11 [§]	—
[#] <i>P</i>	—	—	0.191	0.591	0.511	0.752	—
[§] <i>P</i>	—	—	0.130	0.643	0.677	0.451	—

Note. Values are mean \pm standard deviation. * indicates the comparison of the TT genotype and CC genotype in the VC group; [#] indicates the comparison of the CC genotype in the VC group and NC group; [§] indicates the comparison of the TT genotype and CC genotype in the NC group. Abbreviations: CC, cytosine–cytosine; CT, cytosine–thymine; TT, thymine–thymine; MDA, malondialdehyde; Hcy, homocysteine; PO₂, pressure of oxygen.

of the T allele was 40.5% (Table 1). The frequency of each group of genotypes conformed to the Hardy–Weinberg equilibrium law, the sample was representative of the group (CC (37.0% VS 35.0%), CT (48.0% VS 49.0%), and TT (15.0% VS 16.0%)), and there was no significant difference ($P > 0.05$). The frequency of the T allele mutation in the VC group was slightly lower than that in the NC group (39.0% VS 40.5%), but the difference was not statistically significant ($P = 0.437$). There was no significant difference between varicocele grade II and III (Table 2).

3.3. Test Results of Plasma Homocysteine Levels. The VC and NC groups were divided into three subgroups, CC, CT, and TT, according to the MTHFR C677T genotype. The plasma

Hcy concentrations in the three subgroups of the NC group were 8.65 \pm 4.65, 9.51 \pm 5.77, and 9.68 \pm 4.11 mmol L⁻¹, respectively, and the differences in the data for the CC and TT subgroups were not statistically significant ($P = 0.451$). In the VC group, comparison of the MTHFR genomes showed the lowest Hcy concentration in patients with the CC genotype and the highest in the TT genotype. The plasma Hcy concentrations in the three groups were 8.35 \pm 3.17, 14.79 \pm 7.36, and 19.84 \pm 6.67 mmol L⁻¹, respectively. Statistically significant differences between the data of the CC and TT subgroups were found ($P < 0.001$). However, there was no significant difference in Hcy levels between VC men with the CC genotype and those in the p-NC group ($P = 0.752$), as shown in Table 3 and Figure 2(a).

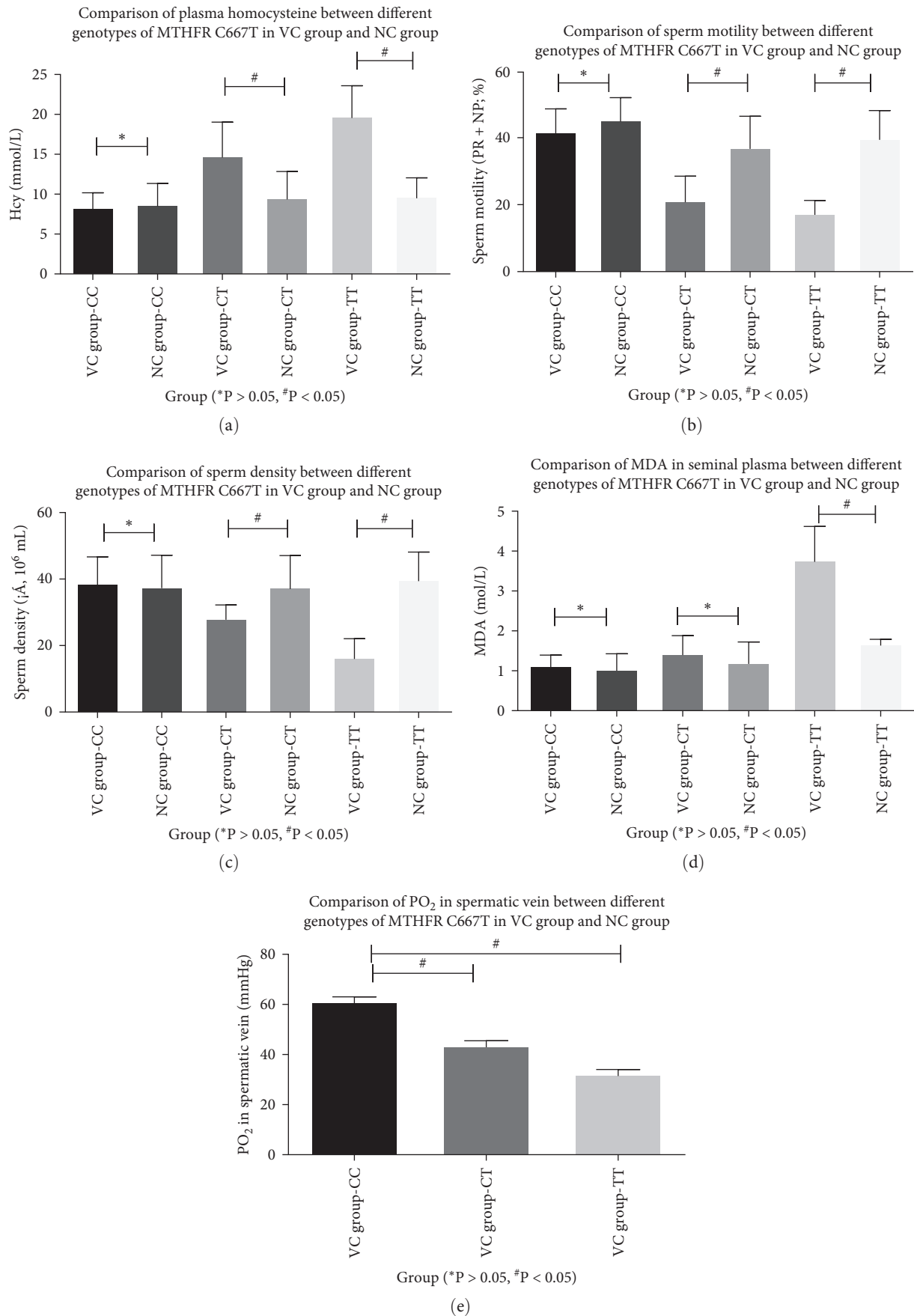


FIGURE 2: Comparison of plasma homocysteine content, sperm motility, sperm density, MDA in seminal plasma, and PO₂ in spermatic vein between different genotypes of MTHFR C667T in VC group and NC group. The difference of plasma homocysteine content, sperm motility, sperm density, and MDA in seminal plasma between the VC-CC and NC-CC groups was not significant ($*P > 0.05$; a-d), while (a) the

VC–CT and TT groups had higher plasma homocysteine content ($^{\#}P < 0.05$) and (b, c) lower sperm motility and sperm density compared to the NC groups ($^{\#}P < 0.05$). (d) MAD content was not significantly different in the VC–CT and NC–CT groups ($^*P > 0.05$), while the CT–TT group was significantly higher than the NC–TT group ($^{\#}P < 0.05$). (e) And for PO_2 levels, VC–CC was significantly higher than both VC–CT and VC–TT groups ($^{\#}P < 0.05$).

3.4. Semen Related Examination Results. The overall data of the VC group were compared with the NC group. There were significant differences in sperm motility (Figure 2(b)), sperm density (Figure 2(c)) and seminal plasma MDA values (Figure 2(d)). However, comparisons between subgroups were slightly different. Patients with the TT genotype in the VC group had decreased sperm viability ($P < 0.001$), decreased sperm density ($P < 0.001$), and increased seminal MDA levels ($P < 0.001$) compared with patients with the CC genotype. However, there were no significant differences in sperm motility, sperm density, and semen MDA levels ($P > 0.05$) between the CC genotype group in the VC group and the CC genotype group in the NC group. Also compared to the CC genotype group of the NC group, the TT genotype group did not show significant differences ($P > 0.05$), as shown in Table 3.

3.5. Results of Spermatic Vein Blood Gas Analysis (BGA) in the VC Operation Group. Compared with that of subjects with the CC genotype, the PO_2 in the spermatic vein of subjects with the TT genotype in the VC group was significantly reduced ($P < 0.001$). Statistical significance is shown in Table 3 and Figure 2(e).

4. Discussion

Previous studies have shown that the MTHFR gene frequently exhibits multiple single nucleotide polymorphisms (SNPs), with the MTHFR C677C >T mutation leading to tetrahydrofolate reductase dysfunction, resulting in elevated Hcy. And Hcy may be associated with the risk of vascular injury and progression [10, 24]. However, further investigation is needed to determine the correlation between MTHFR C677C >T polymorphisms and the pathological changes of spermatic vein [25]. This study examined the correlation between MTHFR gene polymorphism and the risk of patients with grade II–III VC compared to healthy controls. However, our study showed no significant difference in the frequency of MTHFR C677T genotype between the two groups ($P > 0.05$). In contrast, patients with MTHFR C677C >T mutation in the VC group had reduced PO_2 levels in the seminiferous veins, while those with TT genotype had the lowest PO_2 levels ($P < 0.05$). Therefore, we suggest that polymorphisms in the MTHFR gene might not be associated with VC incidence in North China.

MTHFR gene also plays a crucial role in folate metabolism following spermatogenesis. However, studies conducted on different populations have yielded conflicting results regarding the association between MTHFR C677CT polymorphisms and male infertility [26–28]. In our study, patients with VC exhibited higher levels of Hcy and MDA, as well as lower sperm viability and concentration compared to the control group. However, subgroup analysis demonstrated that these differences were

not present in individuals with the CC genotype between the two groups. Patients with the MTHFR C677C >T mutation in the VC group had higher levels of MAD and Hcy, as well as poorer sperm quality, which was particularly significant in those with the TT genotype ($P < 0.05$). Conversely, there was no significant difference observed in the NC group ($P > 0.05$). This finding deviates from previous studies [29] and offers a fresh perspective to further elucidate the existing research contradictions.

Aliakbari et al. [18] and H. Nikzad et al. [30] conducted two meta-analyses on various national and regional population studies, which concluded that the MTHFR C677C >T mutation is significantly associated with the risk of male infertility [18, 30]. Additionally, Aliakbari et al.'s [18] study found a significant association between the MTHFR C677C >T polymorphism and male infertility in the Asian population. In contrast, the findings of Wei et al. [26] were different, especially in the Asian population. These discrepancies may be attributed to the presence of other causative genes, a combination of environmental and disease factors contributing to male infertility, racial disparities, and selection bias. The results of this study suggest that VC may play a key role in the significant association of C677C >T polymorphism with male infertility risk in the MTHFR Asian population.

Oxidative stress is one of the main causes of low sperm quality. Reactive oxygen species (ROS), including superoxide anion (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), hypochlorite ion (OCl^-), etc., together with superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), maintains redox homeostasis in vivo [31]. VC leads to poor blood reflux in the testicular veins. Some hormones or metabolites and hypoxia lead to increased oxygen radical production. In addition, the MTHFR 677 C >T mutation leads to increased levels of Hcy in vivo, which produces a series of reactive oxygen species as described above during its own oxidation. Increased ROS production exceeds the body's clearance rate, disrupting redox homeostasis, generating polyunsaturated fatty acids, and inducing lipid peroxidation such as MDA attack on biofilms, leading to testicular germinal epithelial damage and apoptosis of spermatogonial cells [32]. In the present study, sperm quality was significantly worse in the TT genotype of VC group, but there was no significant change in the TT genotype of NC group and CC genotype of VC group. This might indicate that the VC alone or the MTHFR 677 C >T mutation alone in the present study did not go beyond the regulation of redox homeostasis in the subjects.

This article also has other limitations. First of all, the follow-up of patients with VC infertility after surgery is still ongoing, and the evaluation of surgical outcomes and the relevance of MTHFR C677C >T polymorphism on infertility after the removal of VC as a disease factor need to be

improved. In addition, the sample size of this study is relatively small and the area is limited. We plan to conduct a larger multicenter study in conjunction with other regional medical institutions.

In conclusion, the present study supports the possibility that MTHFR C677C >T polymorphism may be a marker of male infertility susceptibility to VC in North China, without significant correlation with VC occurrence. However, a more comprehensive study is needed to validate our findings.

5. Conclusion

According to our research, MTHFR C677C >T mutation is not associated with the development of varicocele in men in North China, but may affect its progression. It may also lead to poor semen parameters in VC patients by affecting Hcy-related oxidative stress. Larger studies are needed to validate our findings.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Xin Zhongcheng and Feng Yuhong contributed in the conceptualization. Xiu Yiping, Cao Zhiqiang, Yu Dongyang, Yang Caleb, and Wang Jiang contributed in the data curation. Pan Jiancheng contributed in the formal analysis. Pan Jiancheng contributed in the software. Xin Zhongcheng and Feng Yuhong contributed in the funding acquisition. All authors contributed in the Writing—original draft and Writing—review and editing. Cao Zhiqiang and Xiu Yiping these authors contributed equally to this study as co-first authors.

Acknowledgments

The authors thank all patients involved in this study.

References

- [1] E. Babakhanzadeh, M. Nazari, S. Ghasemifar, and A. Khodadadian, "Some of the factors involved in male infertility: a prospective review," *International Journal of General Medicine*, vol. 13, pp. 29–41, 2020.
- [2] C. Krausz, "Male infertility: pathogenesis and clinical diagnosis," *Best Practice & Research Clinical Endocrinology & Metabolism*, vol. 25, no. 2, pp. 271–285, 2011.
- [3] L. R. Ghaleno, A. R. Alizadeh, J. R. Drevet, A. Shahverdi, and M. R. Valojerdi, "Oxidation of sperm DNA and male infertility," *Antioxidants*, vol. 10, no. 1, Article ID 97, 2021.
- [4] P. Birowo, W. Tendi, I. S. Widyahening, W. Atmoko, and N. Rasyid, "The benefits of varicocele repair for achieving pregnancy in male infertility: a systematic review and meta-analysis," *Heliyon*, vol. 6, no. 11, Article ID e05439, 2020.
- [5] V. P. Santana, E. R. James, C. L. Miranda-Furtado et al., "Differential DNA methylation pattern and sperm quality in men with varicocele," *Fertility and Sterility*, vol. 114, no. 4, pp. 770–778, 2020.
- [6] N. Tanji, T. Fujiwara, H. Kaji, S. Nishio, and M. Yokoyama, "Histologic evaluation of spermatic veins in patients with varicocele," *International Journal of Urology*, vol. 6, no. 7, pp. 355–360, 1999.
- [7] D. Tilki, E. Kilic, R. Tauber et al., "The complex structure of the smooth muscle layer of spermatic veins and its potential role in the development of varicocele testis," *European Urology*, vol. 51, no. 5, pp. 1402–1410, 2007.
- [8] Y. Gao, C. Wang, K. Wang, C. He, K. Hu, and M. Liang, "The effects and molecular mechanism of heat stress on spermatogenesis and the mitigation measures," *Systems Biology in Reproductive Medicine*, vol. 68, no. 5–6, pp. 331–347, 2022.
- [9] A. B. Shomarufov, V. A. Bozhedomov, S. I. Giyasov, S. A. Abbosov, and A. A. Kamalov, "Varicocelectomy: a critical analysis of predictors for male reproductive function recovery," *Urologiia*, vol. 6, pp. 148–154, 2020.
- [10] P.-N. Feng, Y.-R. Liang, W.-B. Lin et al., "Homocysteine induced oxidative stress in human umbilical vein endothelial cells via regulating methylation of SORBS1," *European Review for Medical and Pharmacological Sciences*, vol. 22, no. 20, pp. 6948–6958, 2018.
- [11] Y. Yu, C. Jia, Q. Shi, Y. Zhu, and Y. Liu, "Hyperhomocysteinemia in men with a reproductive history of fetal neural tube defects: three case reports and literature review," *Medicine*, vol. 98, no. 2, Article ID e13998, 2019.
- [12] F. Akbarian, M. Tavalaei, M. Dattilio, and M. H. Nasr-Esfahani, "Down-regulated expression of cystathionine β -synthase and cystathionine γ -lyase in varicocele, and infertile men: a case-control study," *Cell Journal*, vol. 24, no. 4, pp. 176–181, 2022.
- [13] W. Feng, Y. Zhang, Y. Pan et al., "Association of three missense mutations in the homocysteine-related MTHFR and MTRR gene with risk of polycystic ovary syndrome in southern Chinese women," *Reproductive Biology and Endocrinology*, vol. 19, no. 1, Article ID 5, 2021.
- [14] T. Xiang, H. Xiang, M. Yan et al., "Systemic risk factors correlated with hyperhomocysteinemia for specific MTHFR C677T genotypes and sex in the Chinese population," *Annals of Translational Medicine*, vol. 8, no. 21, Article ID 1455, 2020.
- [15] F. Liu, J. Du, M. Nie, J. Fu, and J. Sun, "5,10-methylenetetrahydrofolate reductase C677T gene polymorphism and peripheral arterial disease: a meta-analysis," *Vascular*, vol. 29, no. 6, pp. 913–919, 2021.
- [16] K. Ota, T. Takahashi, A. Han, S. Damvaeba, H. Mizunuma, and J. Kwak-Kim, "Effects of MTHFR C677T polymorphism on vitamin D, homocysteine and natural killer cell cytotoxicity in women with recurrent pregnancy losses," *Human Reproduction*, vol. 35, no. 6, pp. 1276–1287, 2020.
- [17] H. Rouhi-Broujeni, B. Pourghesari, and A.-M. Hashemini, "Association of homozygous thrombophilia polymorphisms and venous thromboembolism in Shahrekord, Iran," *Tanaffos*, vol. 15, no. 4, pp. 218–224, 2016.
- [18] F. Aliakbari, F. Pouresmaeli, N. Eshghifard, Z. Zolghadr, and F. Azizi, "Association of the MTHFR 677C>T and 1298A>C polymorphisms and male infertility risk: a meta-analysis," *Reproductive Biology and Endocrinology*, vol. 18, no. 1, Article ID 93, 2020.
- [19] A. Clément, E. Amar, C. Brami et al., "MTHFR SNPs (methyl tetrahydrofolate reductase, single nucleotide polymorphisms) C677T and A1298C prevalence and serum homocysteine levels in >2100 hypofertile caucasian male patients," *Biomolecules*, vol. 12, no. 8, Article ID 1086, 2022.
- [20] A. More, U. Gajbe, O. Olatunji, and B. Singh, "MTHFR gene-polymorphism and infertile men in indian population: a

- systematic literature review,” *Cureus*, vol. 14, no. 7, Article ID e27075, 2022.
- [21] Y. Yang, Y. Y. Luo, S. Wu et al., “Association between C677T and A1298C polymorphisms of the MTHFR gene and risk of male infertility: a meta-analysis,” *Genetics and Molecular Research*, vol. 15, no. 2, 2016.
- [22] T. Fatima, U. Afzal, S. Shaharyar et al., “MTHFR-c 677C>T polymorphism and male infertility: an analysis in a cohort of Pakistani men,” *Revista Internacional de*, vol. 20, no. 4, pp. 274–280, 2022.
- [23] V. B. Ucar, B. Nami, H. Acar, and M. Kiliç, “Is methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism related with varicocele risk?” *Andrologia*, vol. 47, no. 1, pp. 42–46, 2015.
- [24] J. Yu, L. L. Zhang, X. P. Wu et al., “Homocysteine inhibits the viability and migration ability of human umbilical vein endothelial cells by downregulating the expression of vascular endothelial growth factor,” *Experimental and Therapeutic Medicine*, vol. 18, no. 5, pp. 3913–3919, 2019.
- [25] T.-L. Shi, Y. Wu, Y. Li et al., “The relevance of MTHFR C677T, A1298C, and MTRR A66G polymorphisms with response to male infertility in Asians: a meta-analysis,” *Medicine*, vol. 98, no. 8, Article ID e14283, 2019.
- [26] B. Wei, Z. Xu, J. Ruan et al., “MTHFR 677C>T and 1298A>C polymorphisms and male infertility risk: a meta-analysis,” *Molecular Biology Reports*, vol. 39, no. 2, pp. 1997–2002, 2012.
- [27] K. Singh, S. K. Singh, R. Sah, I. Singh, and R. Raman, “Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population,” *International Journal of Andrology*, vol. 28, no. 2, pp. 115–119, 2005.
- [28] H.-C. Lee, Y.-M. Jeong, S. H. Lee et al., “Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility,” *Human Reproduction*, vol. 21, no. 12, pp. 3162–3170, 2006.
- [29] M. K. P. Selvam and A. Agarwal, “Sperm and seminal plasma proteomics: molecular changes associated with varicocele-mediated male infertility,” *The World Journal of Men’s Health*, vol. 38, no. 4, pp. 472–483, 2020.
- [30] H. Nikzad, M. Karimian, K. Sareban, M. Khoshokhan, and A. H. Colagar, “MTHFR-Ala222Val and male infertility: a study in Iranian men, an updated meta-analysis and an in silico-analysis,” *Reproductive BioMedicine Online*, vol. 31, no. 5, pp. 668–680, 2015.
- [31] N. Sadeghi, N. Erfani-Majd, M. Tavalaei, M. R. Tabandeh, J. R. Drevet, and M. H. Nasr-Esfahani, “Signs of ROS-associated autophagy in testis and sperm in a rat model of varicocele,” *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 5140383, 11 pages, 2020.
- [32] L. Micheli, G. Collodel, D. Cerretani et al., “Relationships between ghrelin and obestatin with MDA, proinflammatory cytokines, GSH/GSSG ratio, catalase activity, and semen parameters in infertile patients with leukocytospermia and varicocele,” *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 7261842, 9 pages, 2019.