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ORIGINAL ARTICLE

Erectile Dysfunction

Association between *MTHFR* c.677C>T variant and erectile dysfunction among males attending fertility clinic

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Genetic risk factors have been shown to contribute to the development of sexual dysfunction. However, the role of methylenetetrahydrofolate reductase (*MTHFR*) gene variants in the risk of erectile dysfunction (ED) remains unclear. In this study, we recruited 1254 participants who underwent ED assessed by the International Index of Erectile Function-5. The *MTHFR* c.677C>T variant was also measured by fluorescence polymerase chain reaction (PCR). No significant difference in the genotypic frequency of the *MTHFR* C677T polymorphism (CC, CT, and TT) was observed between men from the ED and non-ED groups. In addition, on binary logistic regression analysis, both crude and adjusted models showed that the risk of ED was not significantly associated with the C677T polymorphism. Interestingly, a significantly higher frequency of the 677TT polymorphism was found in severe and moderate ED (*P* = 0.02). The positive correlation between the *MTHFR* 677TT polymorphism and severe ED was confirmed by logistic regression analysis, even after adjusting for potential confounders (odds ratio [OR] = 2.46, 95% confidence interval [CI]: 1.15–5.50, *P* = 0.02). These findings suggest a positive correlation between the *MTHFR* 677TT polymorphism and the risk of severe ED. Identification of *MTHFR* gene polymorphisms may provide complementary information for ED patients during routine clinical diagnosis.

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Keywords: erectile dysfunction; gene variant; genetic risk factors; methylenetetrahydrofolate reductase; sexual function

INTRODUCTION

Erectile dysfunction (ED) refers to an inability to attain or maintain an erection that is sufficient for satisfactory sexual intercourse.[1] ED is often present in adult men, with a prevalence of 12%–19% in reproductive age.[2] Multiple pathogenetic mechanisms, including vasculogenic, neurogenic, and psychogenic factors, are involved in ED, of which vasculogenic is the most common form.[3] Cardiovascular risk factors such as lifestyle, hypertension, diabetes, and dyslipidemia play a major role in vascular ED.[4] Recently, increased serum homocysteine (Hcy) concentration (hyperhomocysteinemia) has also been associated with ED.[5]

Increasing evidence has shown that genetic risk factors contribute to the development of ED.[6] Thus, identifying genetic risk factors might improve the understanding of the pathogenic molecular basis for ED. The methylenetetrahydrofolate reductase (*MTHFR*) gene, which is localized on chromosome 1 (1p36.3) and is composed of 11 exons, encodes an enzyme that catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.[7] The most common single-nucleotide variant of the *MTHFR* gene is c.677C>T in humans. Each copy of the *MTHFR* 677T allele causes a 35% reduction in *MTHFR* activity under physiological conditions.[8] This mutation occurs in exon 4 of *MTHFR* and causes hyperhomocysteinemia by increasing enzyme thermolability and reducing enzyme activity.[9] A previous study elucidated that hyperhomocysteinemia is an independent predictor of atherosclerosis progression, leading to vascular diseases by affecting both the clotting system and the vascular endothelium.[10] It has been reported that the c.677C>T variant of *MTHFR* is associated with an increased risk of cardiovascular disease, cancer, and male infertility.[11–14] However, little is known about the genetic effect of the *MTHFR* C677T polymorphism on sexual function. Few studies have demonstrated the association between the *MTHFR* c.677C>T variant and ED risk,[6] and the role of the C677T polymorphism in relation to the risk of ED has not been examined in a study with a relatively large sample size.[6] The aim of this study was to investigate the association between the *MTHFR* C677T polymorphism and the risk of ED.

PARTICIPANTS AND METHODS

Participants

A total of 1254 participants from the andrology clinic undergoing fertility evaluation at The First Affiliated Hospital of University of

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Science and Technology of China (Hefei, China) from May 2021 to March 2022 were recruited for this cross-sectional study (**Figure 1**). The participants who completed the questionnaire were included. The exclusion criteria were as follows: (1) men whose age was 50 years or over; (2) men with hypertension, hyperlipidemia, and diabetes; or (3) men with a history of urogenital infections and varicocele. All participants underwent a physical examination by an andrologist. This study was approved by the Ethical Committee of The First Affiliated Hospital of University of Science and Technology of China Ethical Committee (Approval No. 2021-RE-064). Written informed consent was obtained from all subjects before data collection.

Questionnaires and erectile function assessment

All participants in the current study completed a questionnaire that included questions about demographic characteristics (age, height, weight, educational status, and duration of infertility), lifestyle factors (drinking and smoking), and medical history (cryptorchidism, varicoceles, testicular cancer, hypertension, hyperlipidemia, and diabetes). In addition, erectile function was assessed using the Chinese version of the International Index of Erectile Function-5 (IIEF-5) questionnaire, which mainly covers the conditions of erection confidence, erection hardness, maintenance ability, maintenance frequency, and intercourse satisfaction, with the total score ranging from 1 to 25.[15] The IIEF-5 questionnaire was self-completed by each participant to reduce possible influence from physicians. According to the IIEF-5 score, ED was categorized as follows: no symptoms (22–25), mild symptoms (17–21), moderate symptoms (12–16), and severe symptoms (1–11).

MTHFR c.677C>T variant measurements

All participants were asked to provide a venous blood sample at the time of entry into this study. Total genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The *MTHFR* c.677C>T variant (rs1801133) was detected by fluorescence polymerase chain reaction (PCR) according to the manufacturer's protocol (Talde Medical Group, Shenzhen, China).

Statistical analyses

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. According to the IIEF-5 score, two groups were defined, *i.e.*, the ED and non-ED groups. Qualitative variables are reported as frequencies, and quantitative variables are reported as mean ± standard deviation (s.d.). For comparisons between the two groups, Student's *t*-test was used for parametric continuous data, and Pearson's Chi-squared test was used for categorical data. Logistic regression was used to analyze the association between ED and the *MTHFR* C677T polymorphism. Two models were used in this study: a crude model and an adjusted model. The covariates in the adjusted model included age, BMI, drinking, smoking, education, and duration of infertility. All tests were two-sided and performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

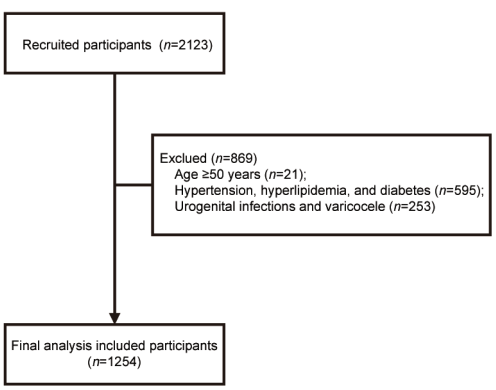

Figure 1: Flowchart of the study population.

RESULTS

A total of 1254 men (mean [s.d.] age: 30.9 [4.4] years) were recruited for this study (**Table 1**). Based on the IIEF-5 score, the participants were divided into two groups: the ED group ($n = 738$) and the non-ED group ($n = 516$). There was no difference in age between the ED group (mean [s.d.] age: 30.9 [4.5] years) and the non-ED group (mean [s.d.] age: 31.0 [4.3] years; $P = 0.76$). BMI was slightly but not significantly higher in the non-ED group. Lifestyle factors, including smoking and drinking, were similar in the two groups. Interestingly, a higher education level was found in the non-ED group ($P < 0.001$), which is in line with the findings of a previous study.[16] In addition, no significant difference in the genotypic frequency of the *MTHFR* C677T polymorphism (CC, CT, and TT) was observed between the ED and non-ED groups ($P = 0.17$). Given that different *MTHFR* enzyme activity levels have been reported among the three *MTHFR* polymorphisms, we further confirmed that the level of serum Hcy was higher in men with the *MTHFR* 677TT polymorphism than that in men

Table 1: Characteristics and descriptive statistics of the whole cohort

Clinical characteristic	*Total participants (n=1254)*	*ED group (n=738)*	*Non-ED group (n=516)*	*P*
Age (year), mean±s.d.	30.9±4.4	30.9±4.5	31.0±4.3	0.76
Age (year), *n* (%)				0.46
<30	499 (39.8)	285 (38.6)	214 (41.5)	
30–39	688 (54.9)	410 (55.6)	278 (53.9)	
≥40	67 (5.3)	43 (5.8)	24 (4.6)	
BMI (kg m^{-2}), mean±s.d.	24.6±3.6	24.4±3.8	24.8±3.4	0.07
Alcohol status, *n* (%)				0.76
Nondrinker	580 (46.3)	344 (46.6)	236 (45.7)	
Drinker	674 (53.7)	394 (53.4)	280 (54.3)	
Smoking status, *n* (%)				0.30
Nonsmoker	695 (55.4)	400 (54.2)	295 (57.2)	
Smoker	559 (44.6)	338 (45.8)	221 (42.8)	
Education level, *n* (%)				<0.001
Primary school	30 (2.4)	29 (3.9)	1 (0.2)	
Junior high school	275 (21.9)	185 (25.1)	90 (17.4)	
High school	216 (17.2)	143 (19.4)	73 (14.2)	
College/university	733 (58.5)	381 (51.6)	352 (68.2)	
Duration of infertility, *n* (%)				0.26
<1 year	606 (48.3)	344 (46.6)	262 (50.8)	
1 year	326 (26.0)	190 (25.8)	136 (26.4)	
2–3 years	172 (13.7)	111 (15.0)	61 (11.8)	
>3 years	150 (12.0)	93 (12.6)	57 (11.0)	
MTHFR C677T polymorphism, *n* (%)				0.17
CC	372 (29.7)	204 (27.7)	168 (32.6)	
CT	602 (48.0)	364 (49.3)	238 (46.1)	
TT	280 (22.3)	170 (23.0)	110 (21.3)	
IIEF-5 score, median (IQR)	21 (18–23)	18 (16–20)	23 (23–24)	<0.001

P values were derived from Pearson's Chi-square test and Student's *t*-test. ED: erectile dysfunction; BMI: body mass index; *MTHFR*: methylenetetrahydrofolate reductase; s.d.: standard deviation; IQR: interquartile range; IIEF-5: International Index of Erectile Function-5; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism

with the 677CC or 677CT polymorphisms (**Supplementary Table 1**), although the number of cases was relatively low. A higher frequency of the *MTHFR* 677T allele was observed in our participants than that in general population from central China[17] (**Supplementary Table 2**). The clinical characteristics of participants stratified by C677T polymorphisms are summarized in **Supplementary Table 3**.

A binary logistic regression analysis for the association between ED and education level, duration of infertility, and *MTHFR* C677T polymorphism is shown in **Table 2**. Compared with the reference group (college/university), men with a lower education level (primary school, junior high school, and high school) had a higher risk of ED (odds ratio [OR] = 1.81, $P < 0.001$; OR = 1.90, $P < 0.001$; OR = 26.79, $P = 0.001$; respectively). In addition, there was no significant association between ED and the duration of infertility ($P = 0.26$). Both crude and adjusted models showed that the risk of ED was not significantly associated with the C677T polymorphism (crude, CT *vs* CC [$P = 0.08$], TT *vs* CC [$P = 0.13$]; adjusted, CT *vs* CC [$P = 0.16$], TT *vs* CC [$P = 0.19$]).

We further investigated the relationship between the frequency distribution of *MTHFR* C677T polymorphisms and levels of ED severity (IIEF-5 score: 1–11, severe; 12–16, moderate; and 17–21, mild). A significantly higher frequency of homozygous 677TT was found in severe and moderate ED ($P = 0.02$; **Table 3**). No significant difference in ED severity was found between men with *MTHFR* 677CT ($P = 0.10$) and 677TT polymorphisms ($P = 0.27$). The positive correlation between the *MTHFR* 677TT polymorphism and severe ED was confirmed by logistic regression analysis, even after adjusting for potential confounders (ED crude, OR = 2.55, 95% confidence interval [CI]: 1.21–5.56, $P = 0.02$; ED adjusted, OR=2.46, 95% CI: 1.15–5.50, $P = 0.02$; **Figure 2**).

Table 2: Logistic regression analyses for erectile dysfunction and education, duration of infertility, and methylenetetrahydrofolate reductase C677T polymorphism

Clinical characteristic	*Crude*		*Adjusted*	
	OR (95% CI)	*P*	*OR (95% CI)*	*P*
Education level				
Primary school	26.79 (5.69–478.4)	0.001	-	-
Junior high school	1.90 (1.42–2.55)	<0.001	-	-
High school	1.81 (1.32–2.50)	<0.001	-	-
College/university	Reference	-	-	-
Duration of infertility				
<1 year	Reference	-	-	-
1–2 years	1.06 (0.81–1.40)	0.66	-	-
2–3 years	1.39 (0.98–1.98)	0.07	-	-
>3 years	1.24 (0.86–1.80)	0.25	-	-
MTHFR C677T polymorphism				
CC	Reference	-	Reference	-
CT	1.26 (0.97–1.64)	0.08	1.21 (0.93–1.59)	0.16
TT	1.27 (0.93–1.75)	0.13	1.24 (0.90–1.71)	0.19

Adjusted for age, BMI, drinking, smoking, education, and duration of infertility. -: no value; BMI: body mass index; *MTHFR*: methylenetetrahydrofolate reductase; OR: odds ratio; CI: confidence interval; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism

Table 3: Frequency distribution of methylenetetrahydrofolate reductase C677T polymorphisms according to the International Index of Erectile Function-5 score

IIEF-5 score range	*Total (n=1254)*	*CC (n=372)*	*CT (n=602)*	*TT (n=280)*
1–11, *n* (%)	61 (4.9)	12 (3.2)	29 (4.8)	20 (7.1)
12–16, *n* (%)	174 (13.9)	50 (13.4)	81 (13.5)	43 (15.4)
17–21, *n* (%)	503 (40.1)	142 (38.2)	254 (42.2)	107 (38.2)
22–25, *n* (%)	516 (41.1)	168 (45.2)	238 (39.5)	110 (39.3)

P values were derived from Pearson's Chi-square test. *P* (CC *vs* CT) = 0.10; *P* (CC *vs* TT) = 0.02; *P* (CT *vs* TT) = 0.27. *MTHFR*: methylenetetrahydrofolate reductase; IIEF-5: International Index of Erectile Function-5; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism

DISCUSSION

ED is one of the most common disorders of male sexuality, affecting millions of adult men all over the world. New-onset ED affects approximately 10% of men of reproductive age and is associated with a significantly increased risk of vasculogenic diseases.[18,19] A recent study showed that *MTHFR* c.677C>T alteration was an independent risk factor for vascular endothelial dysfunction and ED.[10] In the current study, we focused on evaluating the relationship between *MTHFR* C677T polymorphisms and ED severity in a relatively large sample of men attending a clinic for fertility assessment. Our study confirmed that severe ED is significantly associated with the 677TT polymorphism of *MTHFR* but showed no significant association between ED and the *MTHFR* 677CC or 677CT polymorphisms.

Epidemiological studies have reported that the prevalence of ED is more than 50% in men between 40 and 70 years[20,21] and from 15% to 72% in men below 40 years.[22] A meta-analysis showed that the total prevalence of ED in men was approximately 50%, with a prevalence of 20%–40% before the age of 50 years in China.[23] Although the prevalence of ED has an age basis, differences in the patient populations may also explain the differences in the percentages. For example, the prevalence of ED was 42% in nondiabetic young obese men from a primary care-based cohort.[24] Other studies reported a prevalence of 50.8%–61.6% in men in infertile couples using the IIEF-5.[25,26] In this study, approximately 95% of the participants were younger than 40 years of age, and more than 50% of the participants were recruited from the male partners of couples with at least 1 year of infertility. As expected, the ED prevalence was 58.9% in our study population, which is in line with the findings of previous studies using similar enrollment criteria.[25,26] The higher ED prevalence probably reflects an increased

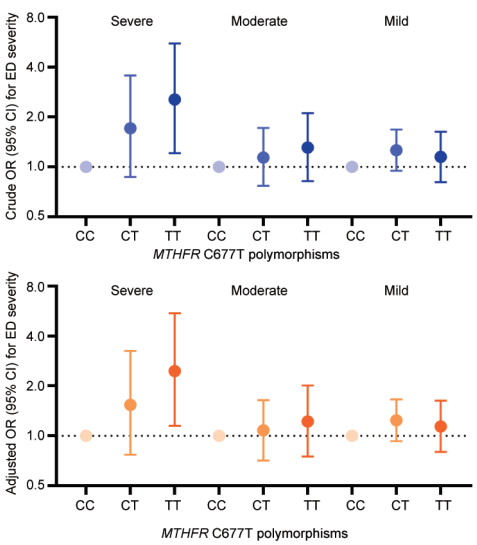

Figure 2: Logistic regression for the association between the *MTHFR* C677T polymorphism and ED severity. ED-adjusted model, adjusted for age, BMI, drinking, smoking, education, and duration of infertility. ED: erectile dysfunction; *MTHFR*: methylenetetrahydrofolate reductase; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism; OR: odds ratio; CI: confidence interval.

risk of sexual dysfunction due to the cohort being recruited from men who seek fertility assessment.[2]

Previous studies have shown that individual differences in human sexual behavior can be heritable.[6,27] The candidate gene approach is widely used to explore the relationship between sexual function and genetic polymorphisms. Notably, the association of several candidate genetic polymorphisms and ED risk has been reported.[6] Endothelial nitric oxide synthase (eNOS) is a key regulator of vascular function, and its G894T polymorphism is associated with an increased risk of ED.[28] Another gene, angiotensin converting enzyme (*ACE*), also plays an essential role in the renin/angiotensin system, and its DD polymorphism (deletion of a 287-bp sequence of DNA in intron 22) was more frequent in men with vascular ED.[29] However, a meta-analysis including 1039 ED patients and 927 healthy controls concluded that the D polymorphism in the *ACE* gene was not associated with an increased risk of ED.[30] In addition to eNOS and ACE, the prevalence of the *MTHFR* 677TT polymorphism is higher in patients with vascular ED than that in those without vascular ED (15.8% *vs* 11.4%).[10] Mutation of *MTHFR* with the C to T substitution at nucleotide 677 causes the impairment of vascular smooth muscle cell and endothelial cell function by increasing plasma Hcy levels and intima-media thickening by decreasing blood flow in the erectile tissue of the corpora cavernosa.[31] The association between another *MTHFR* gene polymorphism (A1298C) and vasculogenic ED was also investigated by Safarinejad *et al.*[10] They found that while the frequencies of AA, AC, and CC did not significantly differ between the ED and non-ED groups, there was a significantly increased risk of severe ED in those with the AC and CC polymorphisms. However, a limitation should be noted that the sample size (114 cases and 228 controls) was not sufficiently large for epidemiological considerations. In contrast to the C677T polymorphism, no significant association was observed between the *MTHFR* polymorphism G1793A and ED risk, although the relationship of the 1793GG polymorphism with elevated Hcy levels has been reported.[10] In this study, with a relatively large sample size, the results showed a positive correlation of the *MTHFR* 677TT polymorphism with severe ED risk, which is in line with data obtained in a previous study.[10] In addition, based on the role of *MTHFR* in Hcy metabolism, the high risk of severe ED may be due to the elevated plasma Hcy concentration by the *MTHFR* 677TT polymorphism.[10] However, another study included a relatively small sample size (90 participants) of middle-aged to elderly (≥42 years, age [mean ± s.d.]: 51.1 ± 7.5 years) men and found that the C677T polymorphism of *MTHFR* was not associated with ED risk but was associated with plasma luteinizing hormone (LH) levels.[32] We assumed that the inconsistent observations of these studies could be explained by the differences in the enrolled populations and sample sizes.

Given that genetic factors are associated with ED risk, the gene polymorphisms in these patients may affect their response to phosphodiesterase type 5 (PDE5) inhibitors. A recent systematic review that included 1308 men with ED from 11 studies concluded that the response to PDE5 inhibitor treatment was significantly associated with gene polymorphisms (*e.g.*, eNOS 786CC polymorphism). Of note, Lombardo *et al.*[33] observed the effective treatment of ED with vitamin B6 and folic acid in young patients with the *MTHFR* 677TT polymorphism who were nonresponsive to PDE5 inhibitors. Although further studies are needed, the identification of *MTHFR* gene polymorphisms might be useful for diagnosing ED in young patients.

Our study has several strengths. First, the mean age of the recruited participants was 30.9 years, reducing the influence of age on the risk of ED. Second, the exclusion criteria in this study included men with hypertension, hyperlipidemia, and diabetes, increasing the independent role of *MTHFR* in the risk of ED. Third, the current study is a large population-based study investigating the association between the *MTHFR* C677T polymorphism and the level of ED severity. Fourth, we also evaluated potential confounders, including age, BMI, lifestyle styles (smoking and drinking), educational status, and duration of infertility, which provides more statistical power.

This study has several limitations. First, this study was a cross-sectional analysis and did not provide a possible direction for the observed associations. Second, only men who were undergoing infertility investigations at a single andrology clinic in central China were selected, and further studies across other parts of China will be needed. Third, we did not analyze the effects of PDE5 inhibitor treatment, although we observed a significant association between the *MTHFR* C677T polymorphism and ED risk. Fourth, metabolic and hormonal parameters (especially the total testosterone level and thyroid function) as well as penile duplex ultrasound findings were not assessed in this study. Finally, recall bias may have occurred in this study when data on the level of ED severity were obtained from the IIEF-5 questionnaire.

CONCLUSIONS

This study indicates a positive correlation between the *MTHFR* 677TT polymorphism and the risk of severe ED. Identification of *MTHFR* gene polymorphisms may provide complementary information for ED patients during routine clinical diagnosis.

AUTHOR CONTRIBUTIONS

SB, MZL, BX and XHJ designed the research study. SB, MZL, YYW, XCH, YXL, BX, and XHJ contributed to the data acquisition. SB, MZL, XHT, THG, LZ, RL, YQZ, PX, BX, and XHJ analyzed the data. SB and MZL wrote the paper. BX and XHJ revised the manuscript and provided comments. All authors have read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

REFERENCES

1 McCabe MP, Sharlip ID, Atalla E, Balon R, Fisher AD, *et al*. Definitions of sexual dysfunctions in women and men: a consensus statement from the Fourth International Consultation on Sexual Medicine 2015. *J Sex Med* 2016; 13: 135–43.
2 Lotti F, Maggi M. Sexual dysfunction and male infertility. *Nat Rev Urol* 2018; 15: 287–307.
3 Thomas JA. Pharmacological aspects of erectile dysfunction. *Jpn J Pharmacol* 2002; 89: 101–12.
4 Terentes-Printzios D, Ioakeimidis N, Rokkas K, Vlachopoulos C. Interactions between erectile dysfunction, cardiovascular disease and cardiovascular drugs. *Nat Rev Cardiol* 2022; 19: 59–74.
5 Salvio G, Ciarloni A, Cordoni S, Cutini M, Muti ND, *et al*. Homocysteine levels correlate with velocimetric parameters in patients with erectile dysfunction undergoing penile duplex ultrasound. *Andrology* 2022; 10: 733–9.
6 Mostafa T, Taymour M. Gene polymorphisms affecting erectile dysfunction. *Sex*

Med Rev 2020; 8: 561–72.
7 Mahbouli S, Dupont C, Elfassy Y, Lameignere E, Levy R. Exploring the potential impact of nutritionally actionable genetic polymorphisms on idiopathic male infertility: a review of current evidence. *Asian J Androl* 2021; 23: 441–9.
8 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, *et al*. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10: 111–3.
9 Santana Bezerra H, Severo de Assis C, Dos Santos Nunes MK, Wanderley de Queiroga Evangelista I, Modesto Filho J, *et al*. The *MTHFR* promoter hypermethylation pattern associated with the A1298C polymorphism influences lipid parameters and glycemic control in diabetic patients. *Diabetol Metab Syndr* 2019; 11: 4.
10 Safarinejad MR, Safarinejad S, Shafiei N. Role of methylenetetrahydrofolate reductase gene polymorphisms (C677T, A1298C, and G1793A) in the development of early onset vasculogenic erectile dysfunction. *Arch Med Res* 2010; 41: 410–22.
11 Rooney M, Hughes CF, Strain JJ, Clements M, McNulty H, *et al*. Impact of the *MTHFR* C677T polymorphism on blood pressure and related central haemodynamic parameters in healthy adults. *J Hum Nutr Diet* 2022; 35: 689–700.
12 Moll S, Varga EA. Homocysteine and *MTHFR* mutations. *Circulation* 2015; 132: e6–9.
13 Gong M, Dong W, He T, Shi Z, Huang G, *et al*. *MTHFR* 677C>T polymorphism increases the male infertility risk: a meta-analysis involving 26 studies. *PLoS One* 2015; 10: e0121147.
14 Aliakbari F, Pouresmaeili F, Eshghifar N, Zolghadr Z, Azizi F. Association of the *MTHFR* 677C>T and 1298A>C polymorphisms and male infertility risk: a meta-analysis. *Reprod Biol Endocrinol* 2020; 18: 93.
15 Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Pena BM. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int J Impot Res* 1999; 11: 319–26.
16 Chen Y, Li J, Li T, Long J, Liao J, *et al*. Association between homocysteine, vitamin B 12, folic acid and erectile dysfunction: a cross-sectional study in China. *BMJ Open* 2019; 9: e023003.
17 Wang X, Fu J, Li Q, Zeng D. Geographical and ethnic distributions of the MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in Chinese populations: a meta-analysis. *PLoS One* 2016; 11: e0152414.
18 Rosen RC, Fisher WA, Eardley I, Niederberger C, Nadel A, *et al*. The multinational Men's Attitudes to Life Events and Sexuality (MALES) study: I. Prevalence of erectile dysfunction and related health concerns in the general population. *Curr Med Res Opin* 2004; 20: 607–17.
19 Caskurlu T, Tasci AI, Resim S, Sahinkanat T, Ergenekon E. The etiology of erectile dysfunction and contributing factors in different age groups in Turkey. *Int J Urol* 2004; 11: 525–9.
20 Levine LA. Erectile dysfunction: a review of a common problem in rapid evolution. *Prim Care Update Ob Gyns* 2000; 7: 124–9.
21 Allen MS. Physical activity as an adjunct treatment for erectile dysfunction. *Nat Rev Urol* 2019; 16: 553–62.
22 Ludwig W, Phillips M. Organic causes of erectile dysfunction in men under 40. *Urol Int* 2014; 92: 1–6.
23 Wang W, Fan J, Huang G, Zhu X, Tian Y, *et al*. Meta-analysis of prevalence of erectile dysfunction in mainland China: evidence based on epidemiological surveys. *Sex Med* 2017; 5: e19–30.
24 Molina-Vega M, Asenjo-Plaza M, Banderas-Donaire MJ, Hernandez-Ollero MD, Rodriguez-Moreno S, *et al*. Prevalence of and risk factors for erectile dysfunction in young nondiabetic obese men: results from a regional study. *Asian J Androl* 2020; 22: 372–8.
25 Song SH, Kim DS, Yoon TK, Hong JY, Shim SH. Sexual function and stress level of male partners of infertile couples during the fertile period. *BJU Int* 2016; 117: 173–6.
26 Khademi A, Alleyassin A, Amini M, Ghaemi M. Evaluation of sexual dysfunction prevalence in infertile couples. *J Sex Med* 2008; 5: 1402–10.
27 Harden KP. Genetic influences on adolescent sexual behavior: why genes matter for environmentally oriented researchers. *Psychol Bull* 2014; 140: 434–65.
28 Gao L, Zhao Z, Guo F, Liu Y, Guo J, *et al*. Association of endothelial nitric oxide synthase polymorphisms with an increased risk of erectile dysfunction. *Asian J Androl* 2017; 19: 330–7.
29 Park JK, Kim W, Kim SW, Koh GY, Park SK. Gene-polymorphisms of angiotensin converting enzyme and endothelial nitric oxide synthase in patients with erectile dysfunction. *Int J Impot Res* 1999; 11: 273–6.
30 Zhang T, Li WL, He XF, Wu ZY, Liu LH, *et al*. The insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme gene and erectile dysfunction risk: a meta-analysis. *Andrology* 2013; 1: 274–80.
31 Lombardo F, Sgro P, Gandini L, Dondero F, Jannini EA, *et al*. Might erectile dysfunction be due to the thermolabile variant of methylenetetrahydrofolate reductase? *J Endocrinol Invest* 2004; 27: 883–5.
32 Sery O, Sramkova T, Klempova J, Stastny F, Lochman J, *et al*. The relationship between the C677T polymorphism of the *MTHFR* gene and serum levels of luteinizing hormone in males with erectile dysfunction. *Neuro Endocrinol Lett* 2012; 33: 499–504.
33 Lombardo F, Tsamatropoulos P, Piroli E, Culasso F, Jannini EA, *et al*. Treatment of erectile dysfunction due to C677T mutation of the *MTHFR* gene with vitamin B6 and folic acid in patients non responders to PDE5i. *J Sex Med* 2010; 7: 216–23.

Supplementary Table 1: Level of homocysteine in participants according to methylenetetrahydrofolate reductase C677T polymorphism

Variable	*MTHFR C677T polymorphism*			P^a	P^b	P^c
	CC (n=4)	*CT (n=8)*	*TT (n=6)*			
Hcy (μmol l^{-1})	9.5±2.3	9.0±1.5	18.6±12.3	0.63	0.19	0.04

[a]*P*, CC *vs* TT; [b]*P*, CC *vs* TT; [c]*P*, CT *vs* TT. *MTHFR*: methylenetetrahydrofolate reductase; Hcy: homocysteine; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism

Supplementary Table 2: Comparison of methylenetetrahydrofolate reductase C677T polymorphism distribution between our study population and the general men in central China (Wang *et al.*[23])

MTHFR C677T polymorphisms	*Our study population, n (%)*	*Central China, n (%)*	*P*
CC	372 (29.7)	15 718 (40.1)	<0.001
CT	602 (48.0)	15 899 (40.6)	
TT	280 (22.3)	7588 (19.3)	
Allele C	1346 (53.7)	47 335 (60.4)	<0.001
Allele T	1162 (46.3)	31 075 (39.6)	

MTHFR: methylenetetrahydrofolate reductase; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism

Supplementary Table 3: Clinical characteristics of participants according to methylenetetrahydrofolate reductase C677T polymorphism

Clinical characteristic	*CC (n=372)*	*CT (n=602)*	*TT (n=280)*	*P*
Age (year), mean±s.d.	31.0±4.1	30.9±4.5	31.1±4.7	0.76
BMI (kg m^{-2}), mean±s.d.	24.3±3.5	24.8±3.8	24.6±3.4	0.18
Alcohol status, *n* (%)				
Nondrinker	178 (47.8)	280 (46.5)	122 (43.6)	0.55
Drinker	194 (52.2)	322 (53.5)	158 (56.4)	
Smoking status, *n* (%)				
Nonsmoker	189 (50.8)	345 (57.3)	152 (54.3)	0.27
Smoker	174 (49.2)	257 (42.7)	128 (45.7)	
Education level, *n* (%)				
Primary school	6 (1.6)	19 (3.2)	5 (1.8)	0.27
Junior high school	69 (18.5)	140 (23.3)	66 (23.6)	
High school	66 (17.7)	98 (16.3)	52 (18.6)	
College/university	231 (62.1)	345 (57.3)	157 (56.1)	
Duration of infertility, *n* (%)				
<1 year	180 (48.4)	292 (48.5)	134 (47.9)	0.99
1 year	98 (26.3)	156 (25.9)	72 (25.7)	
2–3 years	48 (12.9)	85 (14.1)	39 (13.9)	
>3 years	46 (12.4)	69 (11.5)	35 (12.5)	
IIEF-5 score, median (IQR)	21 (18–23)	21 (18–23)	21 (17–23)	0.08

MTHFR: methylenetetrahydrofolate reductase; BMI: body mass index; s.d.: standard deviation; IIEF-5: International Index of Erectile Function-5; IQR: interquartile range; CC: *MTHFR* 677CC polymorphism; CT: *MTHFR* 677CT polymorphism; TT: *MTHFR* 677TT polymorphism