Research Article

MTHFR A1298C Gene Polymorphism and the Risk of Male Infertility in Algerian Population

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ABSTRACT

The purpose of this study was to investigate the association between MTHFR gene single nucleotide polymorphism A1298C and the risk of human male infertility in Algerian population. In this case-control study, peripheral venous blood was taken from 89 patients with spermatogenesis failure and 84 fertile controls for DNA extraction and kept in EDTA vacutainer tubes for molecular analysis. Controls were age-matched to cases**.** The genotyping was carried out using Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) and gel electrophoresis. Data were analyzed using Chi square test. There was no significant difference in genotype frequency of A1298C between cases and controls [cases: AA (59.55 %), AC (38.20 %) and CC (2.25 %); controls: AA (51.19 %), AC (47.62 %) and CC (1.19 %); p= 0.84]. There is no evidence for an association between male infertility and MTHFR 1298C polymorphism in the Algerian population. Future studies with a larger sample size are needed to validate our findings.

Keywords: Algerian population – MTHFR A1298C - polymorphism – male infertility.

INTRODUCTION

Male infertility is a common and complex problem which affects approximately 15% to 20% of couples, and male factor accounting for about half of all cases of infertility.[1,2]

Previous reports have shown that genetic factors play an important role in male infertility.[3] Variants of genes involved in folic acid metabolism pathway, could be associated with the risk of abnormal spermatogenesis. Among genes taking part in folate metabolism, the methylenetetrahydrofolate reductase gene (*MTHFR*) has been the most frequent.[4]

The MTHFR catalyzes the irreversible reduction of 5.10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which acts as a methyl donor for the remethylation of homocysteine into methionine.[5]

Therefore, MTHFR plays an important role in the regulation of DNA/RNA synthesis, methylation, and repair and is closely related with spermatogenesis.[6] The *MTHFR* gene is composed of 11 exons and is located in chromosome 1p36.3.[7,8]

There are two commonly recognized polymorphic variants in the gene encoding for MTHFR: C677T (rs1801133, Ala222Val), and A1298C (rs1801133, Glu429Ala) variant; both are known to decrease the enzyme activity.[7]

The mutation of the MTHFR gene which results in the A1298C polymorphism is located at exon 7 which causes the conversion of glutamine to alanine at codon 429 of the protein and is associated with a 30% decrease in enzymatic activity.[9]

Numerous studies evaluated the relationship between MTHFR gene polymorphisms and male infertility but none of them examined Algerian patients.[4]

Recently, we showed that homozygosity for the 677(C;T) mutation in the MTHFR gene is not a risk factor for idiopathic male infertility[10] and now we aim to assess whether the A1298C polymorphism in the same gene is associated with unexplained reduced sperm quality compared with fertile and/or normozoospermic individuals of Algerian origin.

SUBJECTS AND METHODS

Between October 2008 and May 2015, a total of 173 subjects were enrolled in this case-control study: 59 patients with azoospermia (AZOs), 21 patients with oligoasthenoteratozoospermia (OATs) and 9 patients with asthenozoospermia (ASTs) and 84 unrelated controls (normospermic (n= 68) and fertile "father of at least one child" (n= 16)). All subjects in our analysis were Algerians and were recruited from Ibn Sina Laboratory and Ibn Rochd Clinic.

The patients were selected on the basis of andrological testing, including examination of medical history, semen and hormonal analysis, karyotyping and Y chromosome microdeletions screening. Those with obstructive azoospermia, cryptorchidism, numerical or structural chromosomal abnormalities or microdeletions in the long arm of Y chromosome were excluded from the study.

Ethics Statement

The study was approved by the local Ethical Committee. Each subject gave a sample of his blood after detailed explanation of the purpose of the study; a consent form was also obtained from each subject.

Molecular analysis

Blood samples (7ml) were collected in vacutainer K3EDTA tubes for genomic DNA extraction, which was performed using standard salting out procedure. The purity of DNA was checked by nano drop.

One set of forward 5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3' and reverse 5'-CAC TTT GTG ACC ATT CCG GTT TG-3' primers was used for the amplification of a fragment of 163 bp. For amplification, a volume of 25 ml containing 30 ng genomic DNA, 1.5 mM MgCl2, 200 mM each deoxynucleotide triphosphate, 2 mM each primer, 0.5 U Taq DNA polymerase (Bioline, London, U.K.) and 10X reaction buffer. The PCR conditions were: 4 minutes of initial denaturation at 95°C, followed by 40 cycles of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 4 minutes.

The amplified fragment was digested with 1.U of the MboII restriction enzyme in 37°C for 16 hours. The 1298AA wild-type homozygotes results in five fragments of 56, 31, 30, 28 and 18 bp; the 1298AC heterozygotes genotype produces six fragments of 84, 56, 31, 30, 28 and 18 bp; and the 1298CC homozygotes produces four fragments of 84, 31, 30 and 18 bp. Digestion products were electrophoresed on a 4% agarose gel and visualized under UV light.

Statistical methods

Statistical analyses were performed using Epi info 6.0. Chi square test was used to determine the difference in the genotype and gene frequency. P<0.05 was considered to indicate a statistically significant result.

RESULTS

A total of 89 infertile patients and 84 control subjects were included in this study. Fifty three (59.55%) cases of 89 patients and 43 (51.19%) of controls were 1298AA. Heterozygote genotype was detected in 34 (38.20%) patients and 40 (47.62%) normal controls. Two (2.25%) patients and only one (1.19%) normal control showed 1298CC genotype. These results showed that the population was in the Hardy–Weinberg Equilibrium (HWE) for A1298C genotype.

No significant difference was found in the 1298 SNP between infertile cases and controls in any genetic model (codominant models: AC vs. AA, OR=0.69, 95% CI 0.36-1.33, P=0.29; CC vs. AA, OR=1.62, 95% CI 0.11-64.88, P=0.84; dominant model: AC + CC vs. AA, OR=0.71, 95% CI 0.37-1.36, P=0.34; recessive model: CC vs. AC + AA, OR=0.52, 95% CI 0.02-7.55, P=0.95; allele model: C vs. A, OR=0.81, 95% CI 0.48-1.38, P=0.49) (table 1).

We also investigated possible associations of the polymorphism between controls and the three sub-groups (AZOs, OATs, ASTs) of infertile patients, but the difference was not statistically significant (P> 0.05).

DISCUSSION

Abnormal folate metabolism has been proposed as a factor in male infertility, because folate-derived one-carbon units are involved in DNA synthesis and the regulation of DNA transcription via methylation, two key processes in spermatogenesis.[11]

Several studies have been conducted to determine the association between the A1298C polymorphism in *MTHFR* gene and male infertility.[12] However, the results are conflicting due to differences in the studied populations, various genetic backgrounds, ethnic and geographic variation and different exposures to diverse environmental risk factors.

We carried out a case control study to investigate the role of MTHFR A1298C polymorphism in susceptibility to male infertility in Algerian population. Both patients and fertile controls were from the same common geographical origin. Our results showed that the MTHFR 1298C polymorphism is not a risk factor for male infertility in our population. To our knowledge, this is the first case-control study to evaluate the role of this variant of MTHFR gene in male infertility in Algeria. The results were consistent with a previous report by Park[13], Lee[14], Ravel[15], Montjean[16], Safarinejad[17] and Wei[18] that found no statistical significance for the A1298C variation in unexplained infertile males. Recently and similar to our results, Mfady[19], Kim[20], Kurzawski[9], Li[21] and Ni[6], also found no statistical significance of the MTHFR A1298C polymorphism in infertile males in the Jordanian, Polish and Chinese population, respectively. Another study conducted by Li[22] did not show a clear relationship between the *MTHFR* A1298C polymorphism and male infertility. However, Singh[23], Gava[24], Eloualid[25], have reported that *MTHFR* A1298C may be a genetic risk factor for male infertility. Additionally, the finding of two meta-analysis that were conducted in 2012 including seven case–control studies with 1633 cases and 1735 controls, provided evidence of the association between the MTHFR 1298AC polymorphism and male infertility risk.[4,18] By contrast, after analysis of 2734 cases and 2737 controls by reviewing 10 cases-controls studies published before 2013, Gupta[12] indicated that the same polymorphism is not associated with male infertility risk. Similarly, a stratified analysis on the basis of infertility phenotype did not reveal any significant association between this SNP and azoospermia or OATs.[12]

Folate deficiency is considered as a risk factor for various diseases, including male infertility.[14] Changes in folate status could affect spermatogenesis by causing DNA hypomethylation and inducing uracil misincorporation during DNA synthesis that leads to errors in DNA repair, strand breakage, and chromosomal anomalies.[6] The

hypomethylation of premeiotic germ cells in mouse has been shown to inhibit their differentiation into spermatocytes, it is possible that MTHFR variation in man causes infertility by the same mechanism.[24]

In conclusion, the findings of the current study suggest that genetic polymorphism of MTHFR at A1298C is not a major risk factor for male infertility in our population. The results from this study extend our previous findings that C677T MTHFR polymorphism is not a risk factor for male infertility in Algerian population. To the best of our knowledge, no other study has examined the role of these polymorphisms (C677T, 1298C) on male infertility in Algeria. However, with a limited sample size, especially in subdivided groups, our results allow only preliminary conclusions. Larger studies and functional studies, including data on folate intake and plasma folate levels, are warranted to validate our findings. Moreover, further investigations of other polymorphisms, gene-gene and gene-environment interactions may be helpful to clarify the pathogenesis of the male infertility.

Table 1: Distribution of A1298C polymorphism in MTHFR gene in patients and controls.

	Controls	Patients				OR/P value			
		AZOs	OATs	ASTs	Total	AZOs	OATs	ASTs	Total
	%n	%n	%n	%n	%n				
AA	51.19 43	55.93 33	71.43 15	55.56 5	59.55 53	/	/	/	/
AC	47.62 40	42.37 25	23.81 5	44.44 4	38.20 34	0.81 (0.39-1.69) 0.67	0.36 (0.10-1.19) 0.10	0.86 (0.18-4.05) 0.89	0.69 (0.36-1.33) 0.29
CC	1.19 1	1.69 1	4.76 1	0 0	2.25 02	1.30 (0-49.89) 0.59	2.87 (0-113.58) 0.95	0 (0-185.18) 0.18	1.62 (0.11-46.88) 0.84
AC+CC vs AA	48.81 41	44.06 26	28.57 6	44.44 4	40.44 36	0.83 (0.40-1.70) 0.69	0.42 (0.13-1.30) 0.15	0.84 (0.17-3.95) 0.91	0.71 (0.37-1.36) 0.34
AA+AC vs CC	98.81 83	98.3 58	95.24 20	100 9	97.75 87	0.70 (0.70-26.17) 0.63	0.24 (0.01-9.29) 0.85	P 0.17	0.52 (0.02-7.55) 0.95
Allele A	75.00 126	77.12 91	83.33 35	77.78 14	78.65 40	/	/	/	/
Allele C	25.00 42	22.88 27	16.67 7	22.22 4	21.35 38	0.89 (0.49-1;60) 0.78	0.60 (0.22-1.55) 0.34	0.86 (0.22-3) 0.97	0.81 (0.48-1.38) 0.49

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REFERENCES

1. Hirsh A, Male subfertility, BMJ, 327, 2003, 669–672.
2. Jarvi K, Lo K, Fischer A, Grantmyre J, Zini A, Chow V, Mak V, CUA Guideline: The workup of azoospermic males, Canadian Urological Association journal = Journal de l'Association des urologues du Canada, 4, 2010, 163–167.
3. Miyamoto T, Minase G, Okabe K, Ueda H, Sengoku K. Male infertility and its genetic causes, J Obstet Gynaecol Res, 14, 2015.
4. Shen O, Liu R, Wu W, Yu L, Wang X, Association of the methylenetetrahydrofolate reductase gene A1298C polymorphism with male infertility: a meta-analysis, Ann Hum Genet, 76, 2012, 25-32.
5. Rosenblatt DS, Methylenetetrahydrofolate reductase, Clin Invest Med, 24, 2001, 56–59.
6. Ni W, Li H, Wu A, Zhang P, Yang H, Yang X, Huang X, Jiang L, Lack of association between genetic polymorphisms in three folate-related enzyme genes and male infertility in the Chinese population, J Assist Reprod Genet, 32, 2015, 369–374.
7. Frosst P, Blom H J, Milos R, Goyette P, Sheppard Christal A, Matthews R G, Boers G JH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, and Rozen R, A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase, Nat Genet, 10, 1995, 111–113.
8. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, Chan M, Rozen R, Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR), Mamm Genome, 9, 1998, 652–656.
9. Kurzawski M, Wajda A, Malinowski D, Kazienko A, Kurzawa R, Drozdzik M, Association study of folate-related enzymes (MTHFR, MTR, MTRR) genetic variants with non-obstructive male infertility in a Polish population, Genet Mol Biol, 38, 2015, 42-47.

10. Chellat D, Rezgoune ML, Hamane D, Semmame O, Benlatrèche C, Abadi N, Satta D, Influence of methylenetetrahydrofolate reductase C677T gene polymorphisms in Algerian infertile men with azoospermia or severe oligozoospermia, Genet Test Mol Biomarkers, 16, 2012, 874–878.

11. Singh SK, Sah R, Singh I, Raman R, Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population, Int J Androl, 28, 2005, 115-119.

12. Gupta N, Sarkar S, David A, Gangwar PK, Gupta R, Khanna G, Sankhwar SN, Khanna A, Rajender S, Significant impact of the MTHFR polymorphisms and haplotypes on male infertility risk, PloS One, 8, 2013, e69180.

13. Park JH, Lee HC, Jeong YM, Chung TG, Kim HJ, Kim NK, Lee SH, Lee S, MTHFR C677T polymorphism associates with unexplained infertile male factors, J Assist Reprod Genet, 22, 2005, 361-368.

14. Lee HC, Jeong YM, Lee SH, Cha KY, Song SH, Kim NK, Lee HC, Jeong YM, Lee SH, Cha KY, Song SH, Kim NK, Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility, Hum. Reprod, 21, 2006, 3162-3170.

15. Ravel C, Chantot-Bastaraud S, Chalmey C, Barreiro L, Aknin-Seifer I, Pfeffer J, Berthaut I, Mathieu EE, Mandelbaum J, Siffroi JP, McElreavey K, Bashamboo A, Lack of association between genetic polymorphisms in enzymes associated with folate metabolism and unexplained reduced sperm counts, PLoS One, 4, 2009, e6540.

16. Montjean D, Benkhalifa M, Dessolle L, Cohen-Bacrie P, Belloc S, Siffroi JP, Ravel C, Bashamboo A, McElreavey K, Polymorphisms in MTHFR and MTRR genes associated with blood plasma homocysteine concentration and sperm counts, Fertil Steril, 95, 2011, 635-40.

17. Safarinejad MR, Shafiei N, Safarinejad S, Relationship between genetic polymorphisms of methylenetetrahydrofolate reductase (C677T, A1298C, and G1793A) as risk factors for idiopathic male infertility, Reprod Sci, 18, 2011, 304-15.

18. Wei B, Xu Z, Ruan J, Zhu M, Jin K, Zhou D, Hu Q, Wang Q, Wang Z, MTHFR 677CT and 1298AC polymorphisms and male infertility risk: a meta-analysis, Mol Biol Rep, 39, 2012, 1997-2002.

19. Mfady DS, Sadiq MF, Khabour OF, Fararjeh AS, Abu-Awad A, Khader Y, Associations of variants in MTHFR and MTRR genes with male infertility in the Jordanian population. Gene, 15, 2014, 40-44.

20. Kim SY, Lim JW, Kim JW, Park SY, Seo JT, Association between genetic polymorphisms in folate-related enzyme genes and infertile men with non-obstructive azoospermia, Syst Biol Reprod Med, 21, 2015, 1-7.

21. Li XY, Ye JZ, Ding XP, Zhang XH, Ma TJ, Zhong R, Ren HY, Association between methionine synthase reductase A66G polymorphism and primary infertility in Chinese males. Genet Mol Res, 15, 2015, 3491-500.

22. Li SS, Li J, Xiao Z, Ren AG, Jin L, Prospective study of MTHFR genetic polymorphisms as a possible etiology of male infertility, Genet Mol Res, 13, 2014, 6367-6374.

23. Singh K, Singh SK, Raman R, MTHFR A1298C polymorphism and idiopathic male infertility, J Postgrad Med, 56(4), 2010, 267-279.

24. Gava MM, Chagas Ede O, Bianco B, Christofolini DM, Pompeo AC, Glina S, Barbosa CP, Methylenetetrahydrofolate reductase polymorphisms are related to male infertility in Brazilian men, Reprod Sci, 18, 2011, 1267-72.

25. Eloualid A, Abidi O, Charif M, El Houate B, Benrahma H, Louanjli N, Chadli E, Ajjemami M, Barakat A, Bashamboo A, McElreavey K, Rhaissi H, Rouba H, Association of the MTHFR A1298C variant with unexplained severe male infertility, PLoS One, 7, 2012, e34111.

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