Detection of Polymorphisms in MTHFD1 G1958A and Its Possible Association with Idiopathic Male Infertility

AmirAfshin Khaki[1], Asghar Tanoomand[2], Abolfazl Hajibemani[3,4], Beheshteh Abouhamzeh[3*]

[1] Department of Anatomical sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Department of Basic Sciences, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran

[3] Department of Anatomical Sciences, Faculty of Medicine , AJA University of Medical Sciences,Tehran, Iran

[4] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Review

Keywords: idiopathic; infertility; male; MTHFD1; polymorphism

ABSTRACT

Purpose: The role of male infertility is important in human infertility pathology.

Spermatogenesis is a complex developmental process which is regulated by a number of genes. Methylenetetrahydrofolate dehydrogenase1 (MTHFD1) is involved in the synthesis of purine, pyrimidine, and methionine. The aim of this study was to identify the MTHFD1, G1958A polymorphism and its association with idiopathic male infertility in Iranian population.

Materials and Methods: This case-control study was conducted on 200 Iranian men, 100 cases with idiopathic infertility (experimental group) and 100 normal men (control group). The subjects were assessed for the MTHFD1 G1958A polymorphism, using the polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP). The chi-square test was used to determine the association between MTHFD1 G1958A polymorphism and male infertility, using SPSS software. $P \leq 0.05$ was considered significant.

Results: Totally, the frequency of A allele and AA homozygous genotype was found 51% and 47.3% respectively, with 52.5% and 30% in the experimental group versus 42% and 21% in control group. There was a statistically significant correlation between the frequencies of A allele (95 % CI = 1.028- 2.265, OR = 1.526, $p = 0.035$) and AA homozygous (% CI = 0.995-4.494, OR = 2.114, 95 $p = 0.05$) genotype with the MTHFD1 G1958A polymorphism ($P \leq 0.05$).

Conclusion: These results suggest that the polymorphism in MTHFD1 G1598A gene could be considered as an important genetic disorder associated with the etiology of male infertility.

Review

INTRODUCTION

Infertility is a worldwide problem and has a major impact on the quality of life. Male infertility has an important role in this condition. Infertility of unknown reason is referred to

as idiopathic male infertility. Idiopathic male infertility may be caused by several factors, such as chronic stress and endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities (1,2). Genetic risk factors of male infertility reported in some studies are as follows, Klinefelter's syndrome (3), Deoxyribonucleic acid (DNA) damage by reactive oxygen species and total antioxidant capacity (4), aberrant expression of c-kit (5), poly(ADP-ribose) polymerase-1 (PARP-1), proliferative cell nuclear antigen (PCNA) expression in testicular tissues (6) and Y chromosome microdeletions (7). It has been found that folates and homocysteine are important factors in spermatogenesis (**Figure 1**) (8,9). The disturbance of folate metabolism may lead to spermatozoa DNA damage which reduces semen quality, sperm concentration and motility as well as affecting sperm morphology (10,11). Several enzymes are involved in the One-carbon metabolism pathways, including 5,10-methylenetetrahydrofolate dehydrogenase (MTHFD); 5,10-methenyltetrahydrofolate cyclohydrolase (CH);10-formyltetrahydrofolate synthetase (FS); 5,10-methylenetetrahydrofolate reductase (MTHFR); methionine synthase (MS); serine hydroxymethyltransferase (SHMT) (12). MTHFD1 is a trifunctional cytoplasmic enzyme, which catalyzes the conversion of tetrahydrofolate to the corresponding 10-formyl, 5,10-methenyl, and 5,10-methylene derivatives (figure 1). Genetic disorders like polymorphism within the coding region of MTHFD1 could affect the activity, stability, or level of the enzyme followed by impairment of DNA synthesis and folate metabolism (12). Many polymorphic variants have been found for the genes encoding MTHFD1. To our knowledge, a few studies with controversial findings has been done on the association of the MTHFD1G1958A polymorphism as a genetic risk factor with male infertility (12). No study has been performed to identify the MTHFD1 G1958A polymorphism and its correlation with idiopathic male infertility in Iranian population. The aim of this study was to identify the MTHFD1 G1958A polymorphism and its relationship with idiopathic male infertility in

Review

Iranian population.

MATERIALS AND METHODS

The study was approved by the ethics committee of the AJA University of Medical Sciences (IR.AJAUMS.REC.1396.113).

Study population

The study was conducted on a group of men between the ages 20 and 48 years (mean±SD: 35.0±4.8 years) in Iranian population. A total of 200 men who referred to Qafqaz Fertility Center (Fertility clinic in Ardabil, Iran) were selected for this case-control study, 100 diagnosed with idiopathic infertility and 100 normal subjects. Clinical examination and laboratory tests, including medical history analysis, physical examination and semen analysis were done for those with idiopathic male infertility. The experiment group consisted of men with infertility history of at least one year with their spouses with confirmed normal gynecological assessment (normal transvaginal ultrasound examination, no history of the pelvic inflammatory disease or abdominal operations), after at least one year of regular unprotected sexual intercourse (2-3 times weekly). The exclusion criteria for this study was: the history of prostatitis, urethritis, chromosome abnormality, obstructive lesions, cryptorchidism, varicocele, diabetes mellitus and parotiditis as well as occupational hazards. Analyses of sperm count and motility, vitality, and morphology were performed according to the World Health Organization (WHO) guidelines [20]. The control group was men who had fathered at least one healthy child.

Sample collection

Blood samples (2 ml) taken from each individual were collected in sample tubes containing the anticoagulant, potassium ethylenediaminetetraacetic acid (EDTA K3E 15%, 0.12 ml; BD

Review

Vacutainer, BD Vacutainer Systems, Plymouth, UK). The samples were sent to the laboratory of Maragheh University of Medical Sciences and stored at -20°C for subsequent DNA extraction.

Genomic DNA extraction

Frozen Samples were melted and Vortexed for 10 seconds. DNA was extracted from whole blood using a DNA extraction kit (GeNet Bio, South Korea) according to the manufacturer’s instructions. The DNA was quantified spectrophotometrically, and the integrity assessed via agarose gel electrophoresis (0. 8%). Extracted genomic DNA samples were stored at -20°C in a freezer compartment for subsequent analyses.

Primers

Polymerase chain reaction (PCR) was designed to amplify MTHFD1 DNA fragments, containing G1958A SNPs. Two primers set used for PCR [21] were synthesized by CinnaGen Company, Iran. The MTHFD1 G1958A polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using a forward primer 5'-CCC ACTTTG AAG CAG GAT TG-3'and a reverse 5'-CAT CCC AAT TCC CCT GAT G-3'. Expected length of the produced fragment by primer pair was 232 bp.

Genotyping and PCR-RFLP

We performed PCR in 25 µL reaction volumes containing 10 mM Tris–HCl, pH = 8.4, 50 mM KCl, 1.5 mM MgCl2, 250 µM of each dNTP, 10 pmol of each primer (Cinnagen Inc., Tehran, Iran), and 2.5 U Taq DNA polymerase (Fermentas; Glen Burnie, Maryland) using 4 µL of extracted DNA as template. The PCR thermal cycling conditions were as follows: denaturation for 4 minutes at 94 °C, followed by 35 cycles of 45 seconds at 94 °C, 1 minutes at 58 °C, and 1 minutes at 72 °C with a final extension step of 10 minutes at 72 °C.

Review

Amplification was performed using a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The PCR products were analyzed by agarose gel electrophoresis 1.5% following DNA staining with Safe DNA gel stain and visualized under ultraviolet transillumination. PCR products were digested with 2 µL MspI for 18 hours at 37°C. The digested products were analyzed by agarose gel electrophoresis of 1.5%. Restriction products of 125 bp and 107 bp identified the GG genotype; products of 232 bp, 125 bp, and 107 bp represented the GA genotype; and the 232-bp product represented the AA genotype.

Statistical Analysis

Obtained data were analyzed using the SPSS statistical software (Version 22.0, SPSS Inc, Chicago, Illinois) at the significant level of $P \leq 0.05$. Genotypes distribution and allele frequencies in both groups were determined by direct counting and the correlation of the polymorphism within 1958 position of MTHFD1 gen with idiopathic male infertility was evaluated using chi-square test. Eventually, odds ratios (OR) and 95% confidence intervals (CIs) were calculated for the effects of high risk alleles.

Review

RESULTS

We analyzed the SNPs in MTHFD1 G1598A gene in 100 infertile and 100 fertile men by RFLP-PCR System. PCR amplification of samples of MTHFD1 G1598A is shown in **Figure 2** and Genotyping MTHFD1 G1958A polymorphism by PCR-RFLP is shown in **Figure 3.** GG, GA and AA genotypes represent no polymorphism in MTHFD1 G1958A gene, polymorphism in one allele and in both alleles, respectively.

Genotype and allelic distribution of the G1958A polymorphism have been shown in **table 1**. The polymorphism within MTHFD1 G1958A was found in both groups of experiment and control.

The results of genotypes distribution analysis within MTHFD1 G1598A gene in infertile and

control groups are listed in **Table 2.**

The genotype frequency of the AA compared to GG for the MTHFD1 G1958A polymorphism was significantly higher in experimental group than control group (95 % CI = 0.995- 4.494, OR = 2.114, $p = 0.05$). There was no significant difference between two groups for the GA genotype frequency compared to GG and AA for the MTHFD1 G1958A polymorphism ($P > 0.05$). No significant difference was found between experimental and control groups for GA+AA genotype frequencies compared to that of GG genotype, as well as, for AA compared to GG+GA ($p=0.067$), and GA compared to AA ($P > 0.05$). There was a statistically significant difference between the A allele frequency of infertile patients and control group for the MTHFD1 G1958A polymorphism ($P \leq 0.05$). Taken together, a significant correlation was found in two genetic models of A vs G and AA vs GG and no significant association was found for the other genetic models and when all the eligible studies were pooled together (GA vs GG, GA vs AA, GA+AA vs GG and AA vs. GG+GA) (**Table 2**).

DISCUSSION

In the present study, we evaluated the association of single nucleotide polymorphisms in MTHFD1 G1598A gene as a risk factor of idiopathic male infertility in Iranian population. To our best knowledge, no study has been conducted to date, on the association of polymorphism SNPs in MTHFD1 G1598A gene and the risk of idiopathic male infertility in Iranian population. MTHFD1 is an important gene in folate metabolism pathway. The polymorphism SNPs of this gene has been extensively studied in other diseases. No significant association was found between MTHFD G1258A SNP and spontaneous recurrent abortions [13,14], in some studies. The same result was found for Down syndrome, hypertension and neural tube defects in other studies [15-17] Conversely, a significant

Review

relationship was found between polymorphism of SNP in MTHFD1 gene and neural tube defects in some population [17,18]. Another study showed that MTHFD1 G1958A polymorphism might have a marginally significant association with a decreased risk of cancers [19]. Many studies have been examined the association of folate metabolism genes polymorphisms and male infertility [2,20-25]. Some previous studies have identified MTHFD1 G1958A polymorphism in Iranian population [26,27]. They investigated the association of MTHFD1 G1958A polymorphism with congenital heart defects and Breast Cancer in Iranian Patients [26,27]. The frequency of GG and AA homozygote and GA heterozygote in patients group was (55.9, 30%,) (12.7 and 22.5%) and (31.4 and 48%), respectively, versus (74.5 and 24%), (4.1 and 22%) and (21.4 and 55%) in control group [26,27]. In the present study, the frequency of G and A alleles in infertile men was 47.5% and 52.5% compared to 58% and 42% for corresponding alleles in healthy individuals. The frequency of GG, AA and GA genotypes in infertile group was found 25%, 30% and 45% versus corresponding genotype of control group of 37%, 21% and 42%. In the present study, results showed that the genotype frequency of AA compared to GG for the MTHFD1 G1958A polymorphism in idiopathic infertile patients was significantly different from those in control group. However, the occurrence of polymorphism homozygotes (AA genotype) was significantly higher in the infertile patients compared to control. In addition, there was a significantly higher frequency of A allele in infertile patients than control group for the MTHFD1 G1958A polymorphism. The results of this study indicate that the presence of AA genotype could be considered as a risk factor for idiopathic male infertility in Iranian population. And also, our findings revealed that genotype frequencies of GG and GA were not significantly different between infertile and control groups, and no association between polymorphism SNP in MTHFD1 G1598A gene with male infertility was found for these genotypes. Different results have been reported by different studies regarding the association of SNP in MTHFD1 G1598A gene

Review

with male infertility in different populations[12,28]. In a study conducted on the Romanian population, the reported frequency of G and A alleles in infertile group was 47.72% and 52.27% compared to 54.47% and 45.52% in control group and the frequency of GG and AA and GA in infertile subjects was 27.27%, 31.81% and 40.90% versus 26.86%, 17.91% and 55.22% in control group [29]. In consistent with our finding, they realized that there is an association between MTHFD1 1958AA homozygous genotype and male infertility and a higher rate of polymorphism SNP in MTHFD1 G1598Agene was found in infertile men compared to control group. In another study in the Russian population, [12] the frequency of GG, AA and GA in infertile group was 33.33%, 20.74% and 45.92% in compared to corresponding values of 25.29%, 50.58% and 24.12% in control group. In the same study, genotype GA, genotype AA and allele A showed a reverse association with the risk of azoospermia, indicating the role of polymorphisms in MTHFD1 G1598A in male infertility [12]. Conversely, in a study in the Turkish population, no significant differences were found in the genotype frequencies or the allelic frequencies as well as no relationship between polymorphism SNP in MTHFD1 G1598Agene with male infertility in two groups of infertile and healthy men under the study. They reported the frequency of 58.80% and 41.20% for G and A alleles in infertile men versus 59.60% and 40.40% in control, and the frequency of 32.40%, 14.80% and 52.80 for % GG, AA and GA in infertility group compared to 35.20%, 16.00% and 48.80% in control [28] Findings of their study demonstrated that genotype frequencies or the allelic frequencies were not significantly different between infertility cases and controls and were not any association between polymorphism SNP in MTHFD1 G1598Agene with male infertility [28]. Various results have been obtained regarding the associations of the polymorphism SNP in MTHFD1 (G1598A) gene with male infertility in different populations and studies. The role of polymorphism of other genes of folate pathway in male infertility has been investigated in many studies. Methylenetetrahydrofolate reductase

Review

(MTHFR), methionine synthase (MS) and MS reductase (MTRR) have been found to be critical in DNA synthesis and remethylation [2,30], and there are controversial data on the association of polymorphisms of these genes with male infertility [2,31,32].

Nevertheless, these differences may be arisen from genetic determinants and geographic factors effective in the distribution of the polymorphisms in folate metabolism-related enzyme genes. Other factors including gene-nutrient/environmental and gene racial/ethnic interactions have also been shown to impact on these genetic variants [33].

CONCLUSIONS

In summary, our data provide evidence that the polymorphism SNP in MTHFD1 G1598A gene could be an important genetic factor predisposing to idiopathic infertility in Iranian men. So, folate supplement therapy could be an option for men with idiopathic infertility. However, further studies in different population are needed to confirm the association of different polymorphisms and idiopathic male infertility risk.

Review

ACKNOWLEDGEMENTS

We are grateful to Mr. Mostaphazade for his technical assistance. This work was supported by a grant from AJA University of Medical Sciences of Iran. The authors thank the Maragheh University of Medical Sciences and Qafqaz Fertility Center (Fertility clinic in Ardabil, Iran) for their cooperation.

CONFLICT ON INTEREST

The authors report no conflict of interest.

REFERENCE

1. Dohle G, Colpi G, Hargreave T, et al. EAU guidelines on male infertility. Eur Urol. 2005;48:703-11.
2. Lee H-C, Jeong Y-M, Lee SH, et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. Hum Reprod. 2006;21:3162-70.
3. Akinsal EC, Demirtas A, Ekmekcioglu O. Comparison of the Ratio of the Lenght of the Second and Fourth Digits in Subgroups of Fertile and Infertile Cases. Urology journal. 2017;14:3081-4.
4. Vatannejad A, Tavilani H, Sadeghi MR, Amanpour S, Shapourizadeh S, Doosti M. Evaluation of ROS-TAC score and DNA damage in fertile normozoospermic and infertile asthenozoospermic males. Urology journal. 2017;14:2973-8.
5. Jin G, Liu J, Qin Q, et al. Increased Level of c-kit in Semen of Infertile Patients with Varicocele. Urology journal. 2017;14:3023-7.
6. Akarsu S, Büke B, Gürgen SG, et al. Differences in Poly (ADP-ribose) Polymerase1-(PARP1-) and Proliferative Cell Nuclear Antigen (PCNA) Immunoreactivity in Patients Who Experienced Successful and Unsuccessful Microdissection Testicular Sperm Extraction Procedures. Urology journal. 2017;14:5018-22.
7. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. Reprod Biomed online. 2008;16:504-13.
8. Boxmeer JC, Smit M, Weber RF, et al. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. J Androl. 2007;28:521-7.
9. Crha I, Kralikova M, Melounova J, et al. Seminal plasma homocysteine, folate and cobalamin in men with obstructive and non-obstructive azoospermia. J Assist Reprod Genet. 2010;27:533-8.
Review

10. Lewis S, Aitken R. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res. 2005;322:33-41.
11. Saleh RA, Agarwal A, Nada EA, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril. 2003;79:1597-605.
12. Weiner AS, Boyarskikh UA, Voronina EN, et al. Polymorphisms in folate-metabolizing genes and risk of idiopathic male infertility: a study on a Russian population and a meta-analysis. Fertil Steril. 2014;101:87-94. e3.
13. Crişan TO, Trifa A, Farcaş M, et al. The MTHFD1 c. 1958 G> A polymorphism and recurrent spontaneous abortions. J Matern Fetal Neonatal Med. 2011;24:189-92.
14. Kim JH, Jeon YJ, Lee BE, et al. Association of methionine synthase and thymidylate synthase genetic polymorphisms with idiopathic recurrent pregnancy loss. Fertil Steril. 2013;99:1674-80. e3.
15. Fowdar JY, Lason MV, Szvetko AL, Lea RA, Griffiths LR. Investigation of homocysteine-pathway-related variants in essential hypertension. Int J Hypertens. 2012;2012.
16. Neagos D, Cretu R, Tutulan–Cunita A, Stoian V, Bohiltea LC. Methylenetetrahydrofolate dehydrogenase (MTHFD) enzyme polymorphism as a maternal risk factor for trisomy 21: a clinical study. J Med Life.. 2010;3:454.
17. Zheng J, Lu X, Liu H, Zhao P, Li K, Li L. MTHFD1 polymorphism as maternal risk for neural tube defects: a meta-analysis. Neuro Sc. 2015;36:607-16.
18. Hol FA, Put NM, Geurds M, et al. Molecular genetic analysis of the gene encoding the trifunctional enzyme MTHFD (methylenetetrahydrofolate-dehydrogenase, methenyltetrahydrofolate-cyclohydrolase, formyltetrahydrofolate synthetase) in patients with neural tube defects. Clin Genet. 1998;53:119-25.

Review

19. Zhang H, Ma H, Li L, Zhang Z, Xu Y. Association of methylenetetrahydrofolate dehydrogenase 1 polymorphisms with cancer: a meta-analysis. PLoS One. 2013;8:e69366.

20. Aston KI, Krausz C, Laface I, Ruiz-Castane E, Carrell DT. Evaluation of 172 candidate polymorphisms for association with oligozoospermia or azoospermia in a large cohort of men of European descent. Hum Reprod. 2010;25:1383-97.

21. Park JH, Lee HC, Jeong Y-M, et al. MTHFR C677T polymorphism associates with unexplained infertile male factors. J Assist Reprod Genet. 2005;22:361-8.

22. Stuppia L, Gatta V, Scarciolla O, et al. The methylenetethrahydrofolate reductase (MTHFR) C677T polymorphism and male infertility in Italy. J Endocrinol Invest. 2003;26:620-2.

23. Ebisch IM, van Heerde WL, Thomas CM, van der Put N, Wong WY, Steegers-Theunissen RP. C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. Fertil Steril. 2003;80:1190-4.

24. Murphy LE, Mills JL, Molloy AM, et al. Folate and vitamin B12 in idiopathic male infertility. Asian J. Androl. 2011;13:856.

25. Dohle G, Halley D, Van Hemel J, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod. 2002;17:13-6.

26. Khatami M, Ratki FM, Tajfar S, Akrami F. Relationship of the MTHFD1 (rs2236225), eNOS (rs1799983), CBS (rs2850144) and ACE (rs4343) gene polymorphisms in a population of Iranian pediatric patients with congenital heart defects. Kaohsiung J Med Scis. 2017;33:442-8.

27. Rasti M, Tavasoli P, Monabati A, Entezam M. Association between HIC1 and RASSF1A promoter hypermethylation with MTHFD1 G1958A polymorphism and

Review

clinicopathological features of breast cancer in Iranian patients. Iran Biomed J. 2009;13:199.

28. Balkan M, Atar M, Erdal ME, et al. The possible association of polymorphisms in MTHFR, MTRR, and MTHFD1 genes with male infertility. Int Med J. 2013;20:404-8.

29. Popp RA, Farcas MF, Trifa AP, Militaru MS, Crisan TO, Pop IV. The Methylenetetrahydrofolate Dehydrogenase (MTHFD 1) gene G1958A polymorphism and idiopathic male infertility in a Romanian population group. Clujul Medical. 2012;85:54-8.

30. 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. 2015;38:42-7.

31. Bezold G, Lange M, Peter RU. Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. N Engl J Med. 2001;344:1172-3.

32. 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. 2012;76:25-32.

33. Toffoli G, De Mattia E. Pharmacogenetic relevance of MTHFR polymorphisms. 2008.
Review

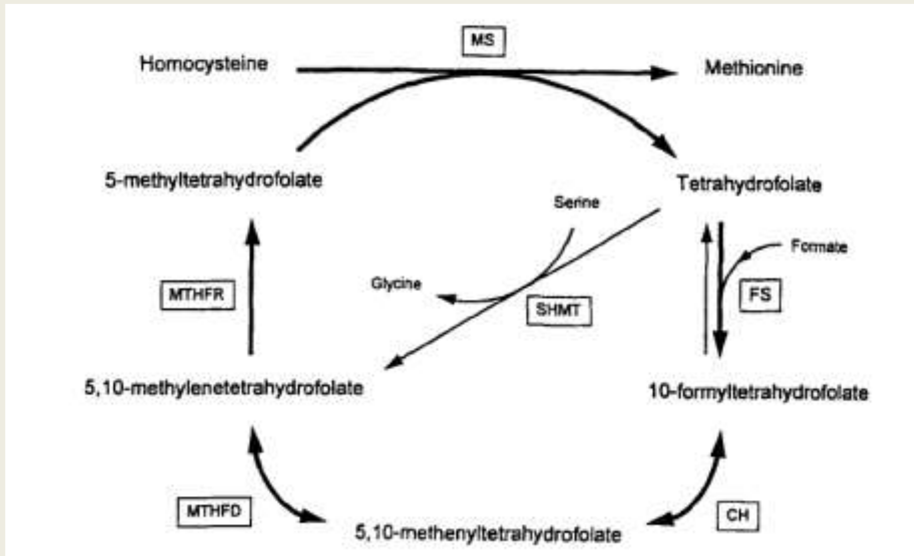


Figure 1. Folate-mediated one-carbon metabolism.

MTHFD, 5,10-methylenetetrahydrofolate dehydrogenase; CH, 5,10-methenyltetrahydrofolate cyclohydrolase; FS, 10-formyltetrahydrofolate synthetase; MTHFR, 5,10-methylenetetrahydrofolate reductase; MS, methionine synthase; SHMT, serine hydroxymethyltransferas

Review

Figure 2. Agarose gel electerophoresis after PCR amplification of samples of MTHFD1 G1598A. “M” represents marker 100bp. The PCR product size was 232bp.

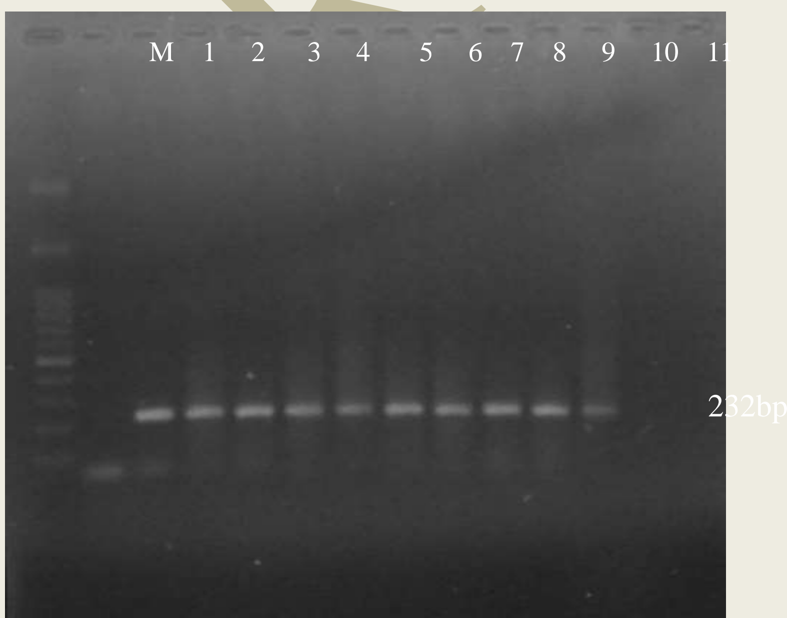
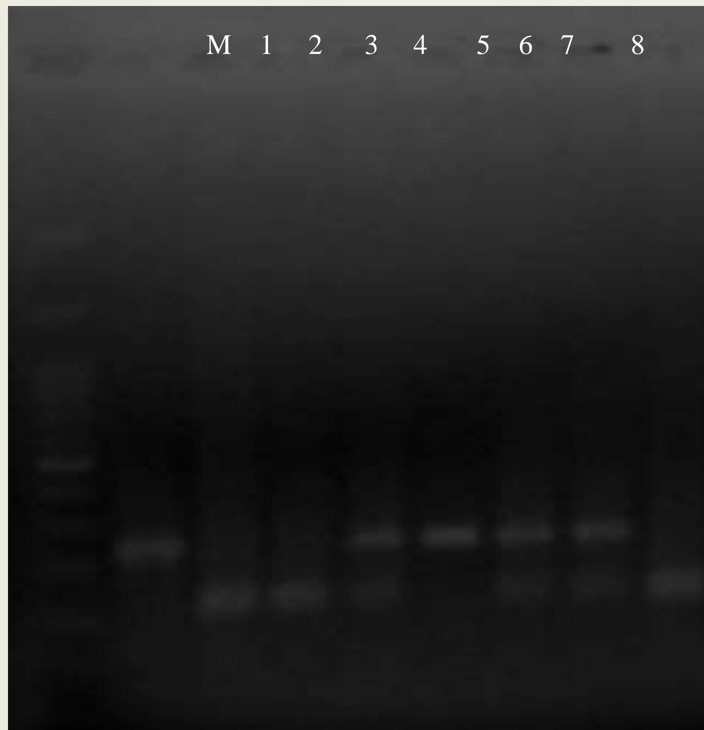


Figure 3. Genotyping MTHFD1 G1958A polymorphism by PCR-RFLP. M: marker 100bp. lane 1, 5: shows a 232 bp band indicating the homomutant type allele (AA genotype); Lane 2, 3 and 8: show 125 and 107bp bands denoting wild type allele (GG genotype); Lane 4, 6 and 7: show 232, 125 and 107bp bands denoting heteromutant type allele (GA genotype).



Review

Table 1 Allele and genotype frequencies for MTHFD1G1958A in infertile and control groups.

	Alleles frequencies, n (%)		**Mutation type n (%)**		
	G allele	A allele	GG	GA	AA
infertility (n=100)	95 (47.5)	105 (52.5)	25 (25)	45 (45)	30 (30)
Controls (n=100)	116 (58)	84 (42)	37 (37)	42 (42)	21 (21)
Total (200)	211 (52.7)	189 (47.3)	62 (62)	87 (87)	51 (51)

Table 2. Genotypes distribution analysis in infertile and control groups.

MTHFD1 G1958A (infertility cases –controls)	**Odds ratio**	**P-value**	**(95% CI)**
A vs G	1.526	0.035	1.028- 2.265
AA vs GG	2.114	0.05	0.995- 4.494
GA vs GG	1.586	0.169	0.820- 3.065
GA vs AA	1.333	0.480	0.633- 2.681
GA/AA vs GG	1.762	0.067	0.959- 3.236
AA vs. GG/GA	1.612	0.144	0.847- 3.069

Review