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Methionine synthase A2756G transition might be a risk factor for male infertility: Evidences from seven case-control studies

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ABSTRACT

Methionine synthase (MTR) has a crucial role in DNA synthesis and methylation reactions. The aim of this study was to investigate the association of the *MTR*-A2756G polymorphism with idiopathic male infertility. Blood samples were collected from 217 idiopathic infertile- and 233 healthy-men, and *MTR*-A2756G genotyping was performed by PCR-RFLP. Meta-analysis was conducted by pooling our data with the data obtained from 6 previous studies. Also, the effects of this substitution on protein structure were evaluated by bioinformatics tools. Our study revealed the association of AG-genotype, GG-genotype, and G-allele with male infertility. Meta-analysis showed a significant association between A2756G transition and male infertility. In addition, structural analysis of the transition effect on protein revealed a significant influence on MTR function (with score: 38; expected accuracy: 66%). These findings suggest that the A2756G substitution might be a genetic risk factor and a potential biomarker for idiopathic male infertility.

1. Introduction

Spermatogenesis abnormalities are the main cause of male infertility. There are several genetic factors, involving in different pathways which disrupted spermatogenesis (Chuma et al., 2009). Folate metabolism pathway composed of several genes which their products are essential for RNA, DNA and protein synthesis and methylation modification (Crider et al., 2012). Therefore, this pathway plays a crucial role in the preservation of genome integrity and imprinting (Fowler, 2005). Also, the aberrant function of folate pathway could results in harmful situation. Homocysteine accumulation as an output of such failure may affect development of gametes by inhibiting the production of nitric oxide, a crucial element in sperm capacitation and acrosome reaction (Duthie et al., 2013; Herrero et al., 2003; Jacob et al., 1998; Stuhlinger et al., 2001).

DNA damage in sperm may affect sperm parameters including: motility, count and morphology (Lewis and Aitken, 2005; Singh and Jaiswal, 2013). Changes in methylation of DNA and histone proteins can increase genome destabilization and lead to failure of epigenetic controls in proliferation (Godmann et al., 2009).

Three key enzyme: methylenetetrahydrofolate reductase (*MTHFR*); methionine synthase reductase (*MTRR*); and methionine synthase (*MTR or MS*) regulate folate metabolism (Chen et al., 2001; Gava et al., 2011). Methylenetetrahydrofolate reductase catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Methionine as a precursor for S-adenosylmethionine, is produced via the transfer of a methyl group from 5-methyltetrahydrofolate. The reaction is catalyzed by methionine synthase and methionine synthase reductase. Methionine synthase catalyzes conversion of homocysteine to methionine (Fig. 1). In animals, MTR function is essential for maintaining adequate intracellular folate pools and it requires vitamin B12 as a cofactor (Leclerc et al., 1996).

The *MTR* gene contains 33 exons, which is located on chromosome 1 (1q43). There is a common polymorphism (A2756G; rs1805087) in exon 26 at position 2756 of the *MTR* gene (Fig. 2A) that results in a transition of adenine to guanine nucleotide. It may be influence the enzyme activity because of amino acid substitution

Abbreviations: HWE, Hardy–Weinberg equilibrium; MTR, Methionine synthase; NCBI, National Center for Biotechnology Information; NOA, Non-Obstructive Azoospermia; nsSNP, Non-synonymous Single Nucleotide Polymorphism; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; pI, Isoelectric Point; SNAP, Screening for Non-Acceptable Polymorphisms; SNP, Single Nucleotide Polymorphism; WHO, World Health Organization.

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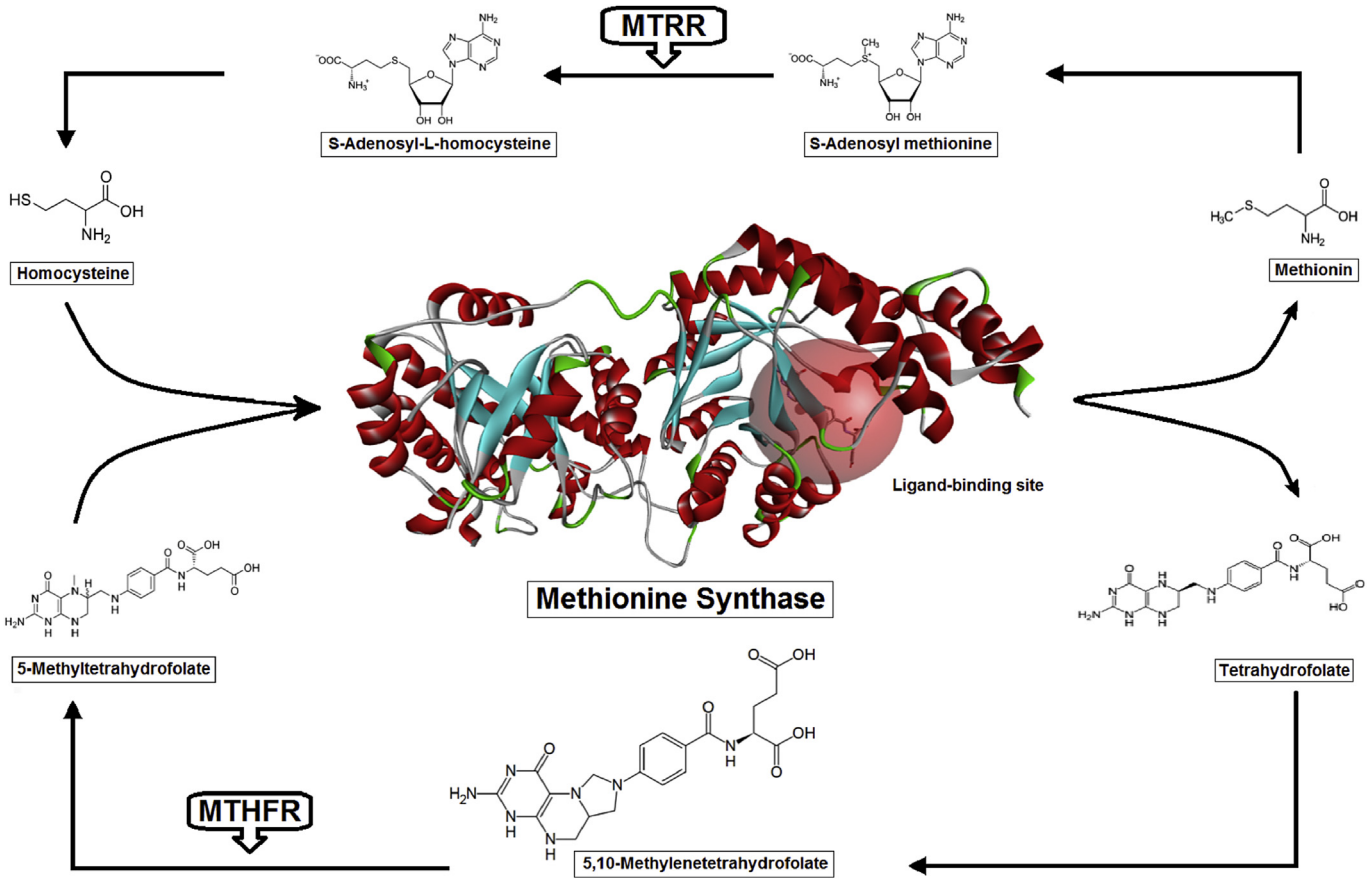

Fig. 1. Role of MTR in folate metabolism. MTR catalyzes the transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine, and produces THF and methionine; MTHFR reduces methylenetetrahydrofolate to methyltetrahydrofolate; MTRR catalyzes the reductive methylation of MTR, which keeps active the MTR during the folate cycle.

in translated protein (Matsuo et al., 2001). The aim of this study was to investigate the association of *MTR*-A2756G transition with male infertility in a case-control study which is followed by a meta- and a bioinformatics-analysis.

2. Materials and methods

2.1. Subjects, sample collection, and DNA isolation

The blood and semen samples of the 217 idiopathic infertile men [103 non-obstructive azoospermia (NOA); 114 oligozoospermia] and 223 age-matched fertile men were recruited from the infertility clinics of Fatemeh Zahra Hospital (Babol, Iran). The mean age of infertile men was 32.26 ± 5.13 years, and of fertile subjects was 33.24 ± 5.71. The subjects with known causes of infertility were excluded. Therefore, the patients with history of cryptorchism, orchitis, obstruction of the vas deferens, varicocele, infectious diseases, drug abuse, diabetes mellitus, abnormal hormon profile (LH, FSH, and testosterone), Y-chromosome microdeletion and abnormal karyotype were excluded from the study. So only men with idiopathic infertility were included in the study. In addition, since the *MTR*-A2756G is a genetic risk factor for various genetic and familial diseases such as neurodegenerative diseases (Beyer et al., 2003), cardiovascular diseases (Chen et al., 2012; Zhang and Dai, 2001), head and neck cancer (Galbiatti et al., 2010), gastric cancer risk (Kim et al., 2016), and some other cancers (Yu et al., 2010), we screened the cases and controls for aforementioned diseases. Then we excluded subjects with history of any genetic and familial disorders. The control group was composed of 223 fertile men with normal sperm parameters, without history of infertility and at least one offspring.

Semen samples analyzed according to the World Health Organization guidelines (WHO, 1999) and the subjects with sperm concentration< 20 million/ml, progressive motility< 50%, and normal forms< 14% classified as oligozoospermic, asthenozoospermic, and teratozoospermic, respectively. In addition the patients without spermatozoa in the ejaculate semen were considered as azoospermic.

Blood samples were collected from fertile and infertile men into sterile tubes containing sodium citrate. Total genomic DNA was isolated from blood samples by DNG plus DNA extraction buffer (Cinnagen, Iran). Written informed consent obtained from all subjects. The study approved by the Medical Ethics Committee of Babol University of Medical Sciences.

2.2. MTR-A2756G genotyping

The *MTR*-A2756G polymorphism was analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). *MTR* fragment was amplified by thermocycler (Eppendorf Co., Germany) using two primers: *MTR*f (5′-AAGCCCACTGAGTTTACCTTTTC) and *MTR*r (5′-ATCCAAAGCCTTTTACACTCCTC) as forward and reverse primers, respectively. Amplification of *MTR* fragment performed in 25 μl PCR mixture containing 60 ng of template DNA, 0.2 μl *Taq* DNA polymerase, 0.5 μl dNTPs mix, 0.35 μM forward primer, 0.35 μM reverse primer, and 1.5 μM $MgCl_2$. To screening of A2756G transition, PCR products were digested by *Hae*III restriction enzyme by incubating overnight at 37 °C. The