Gene 536 (2014) 40–44

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Associations of variants in *MTHFR* and *MTRR* genes with male infertility in the Jordanian population

Doaa S. Mfady [a], May F. Sadiq [a,*], Omar F. Khabour [b], AbdulFattah S. Fararjeh [b], Aymen Abu-Awad [c], Yousef Khader [d]

[a] Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid 22110, Jordan
[b] Department of Medical Laboratory Sciences, Jordan University of Science and Technology, Irbid 22110, Jordan
[c] AL-Hussein Medical City Hospital, Amman, Jordan.
[d] Department of Public Health, Jordan University of Science and Technology, Irbid 22110, Jordan

ARTICLE INFO

Article history:
Accepted 2 December 2013
Available online 12 December 2013

Keywords:
MTHFR
MTRR
Single nucleotide polymorphism
Jordan
Infertility
Male

ABSTRACT

Folate pathway is expected to play an important role in spermatogenesis since it is involved in DNA synthesis, repair and methylation. The purpose of this study was to examine the association between male infertility and the *MTHFR* (C677T and A1298C) and *MTRR* (A66G) polymorphisms. A group of 300 males was recruited in this study from different Jordanian infertility clinics. Of these, 150 cases of infertile men that included oligozoospermia cases (n = 45), severe oligozoospermia (n = 71) and azoospermia (n = 34) were studied. The other 150 males were age matched fertile controls. Genotyping of *MTHFR* and *MTRR* polymorphisms was performed using PCR-RFLP technique. The results showed an association between *MTHFR* 677TT genotype and male infertility ($P < 0.05$). However, the distribution of *MTHFR* A1298C and *MTRR* A66G genotypes were not different between the fertile and infertile groups ($P > 0.05$). In addition, none of the examined polymorphisms was related to any of the semen parameters in the infertile group. In conclusion, this study showed that *MTHFR* C677T polymorphism is associated with male infertility in Jordanians.

1. Introduction

About 10 to 15% of married couples worldwide suffer from infertility in which 50% of it was attributed to the male partners (Sharma et al., 2004). The defect in spermatogenesis ranges from azoospermia with absence of mature germ cells to oligozoospermia with formation of low number of sperms. The causes of infertility in a considerable fraction of infertile male are unknown (Greenberg et al., 1978; Pryor et al., 1997), and were suggested to be complex due to the fact that the network of genes implicated in spermatogenesis and its regulation are located on different autosomes as well as on the sex chromosomes. In addition, male infertility is associated with different environmental factors such as metabolic syndrome, obesity, and diet (Agbaje et al., 2007; Stillman et al., 1986; Teerds et al., 2011). A significant portion of the men infertility cases reported in different countries was hereditary, while the rest were proven unrelated to genetic factors (Greenberg et al., 1978; Tas et al., 1996).

The folate metabolic pathway may play an important role in spermatogenesis since folate deficiency is associated with hyperhomocysteinemia that is considered as a risk factor for male infertility (Altmae et al., 2010). Folate is also related to DNA synthesis and methylation (Forges et al., 2008; Ravel et al., 2009; Wu et al., 2010), therefore, variations in the genes encoding key enzymes involved in folate metabolism such as methylene tetrahydrofolatereductase (*MTHFR*) and methionine synthase reductase (*MTRR*) could be potential risk factors for infertility in men (Young et al., 2008). Two variants of *MTHFR* (C677T: rs1801133 and A1298C rs1801131) and one variant of *MTRR* (A66G; rs1801394) have clinical significance. *MTHFR* C677T is associated with different disorders, including cardiovascular and neuronal diseases and some cancers (Galbiatti et al., 2012; Marosi et al., 2012; van der Linden et al., 2006), while *MTHFR* A1298C is associated with cancer, pregnancy loss and hypertension (Alghasham et al., 2012; Lee and Song, 2010; Qin et al., 2013; Yan et al., 2012). In addition, the *MTRR* A66G polymorphism alters the enzyme activity significantly and is associated with different disorders including neural tube defects, colorectal cancer and coronary artery diseases (Gueant-Rodriguez et al., 2005; Ouyang et al., 2013; Zhou et al., 2012).

The associations of MTHFR and MTRR polymorphisms with male infertility were examined in several populations from Asia, Europe and America (Gupta et al., 2011; Wei et al., 2012; Wu et al., 2012). However, such information was lacking from the Arab populations. In this study, the associations between male infertility and each of the three

Abbreviation: CI, confidence interval; DNA, deoxyribonucleic acid; EDTA, ethylene diamine tetra-acetate; IVF, in vitro facilitated; *MTHFR*, methylene tetrahydrofolatereductase; *MTRR*, methionine synthase reductase; OR, odd ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length Polymorphism; SNP, single nucleotide polymorphism.

* Corresponding author at: Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid 21163, Jordan. Tel.: +962 077 617310; fax: +962 2 7211117.
E-mail addresses: may@yu.edu.jo, maysadiq333@yahoo.com (M.F. Sadiq).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.12.001

polymorphisms *MTHFR* C677T and A1298C, and *MTRR* (A66G) have been examined in the Arab Jordanian population.

2. Materials and methods

2.1. Subjects and collection of blood samples

A case group of 150 infertile men with oligozoospermia (n = 45), sever oligozoospermia (n = 71) and azoospermia (n = 34) were recruited from two major IVF clinics in Jordan: King Abdullah University Hospital (Irbid, northern part of Jordan) and AL-Hussein Medical City Hospital (Amman, central part of Jordan). Patients with recognizable causes of male infertility such as chromosomal abnormalities, hypogonadotropic hypogonadism, infections and obstructive azoospermia were not included in the study (Khabour et al., 2013). Age and geographical origin-matched controls of 150 fertile men were randomly recruited from the same hospitals and IVF clinics. Fertile control men were defined as those who had fathered a child and their semen analyses were >20 million sperms/mL. Clinical and demographic characteristics of all subjects were collected using a questionnaire that covered fertility parameters, medical history, chronic diseases and social habits. A written consent was obtained from each participant and the work was conducted in accordance with the declaration of Helsinki (World Medical Association Declaration of Helsinki, 2000). Blood samples (3 mL) were collected in EDTA tubes from participants for genomic DNA extraction, which was performed using a commercial kit according to the manufacturer instruction (Gentra Puregene Blood, Germany).

2.2. Genotyping of MTHFR and MTRR SNPs

MTHFR C677T, *MTHFR* A1298C and *MTRR* A66G genotypes were determined by PCR amplification and subsequent digestion with *Hinf* I, *Mbo* II and *Nsp* I (New England Biolabs, Ipswich, MA, USA, Fig. 1) restriction enzymes respectively (Szvetko et al., 2007; Yi et al., 2002). Table 1 summarizes the genotyping conditions and requirements, which include the primers for DNA amplification (Integrated DNA Technology, USA), PCR conditions and size of products, restriction conditions and the sizes of the digested DNA fragments. For amplification of each SNP, 25 μL reactions were used containing 5 ng of genomic DNA, 1 μM of each primer and PCR master mix (Promega, Madison, USA). Restriction reactions were performed according to the manufacturer instructions (New England Biolabs, Ipswich, MA, USA). Amplified and restricted DNA fragments were resolved by electrophoresis on 3% high resolution agarose and visualized under UV light after staining with ethidium bromide (Sigma-Aldrich, USA).

2.3. Statistical analysis

Statistical analysis was performed using the statistical package for social studies SPSS. Chi-square test was used to evaluate the genotype distribution and allele frequencies of the studied polymorphisms, and whether individual variants were in the Hardy–Weinberg equilibrium (HWE) at each locus in the samples. Power calculation was computed using an online sample size calculator (OSSE). Linkage disequilibrium and haplotype frequencies were calculated using the SHEsis program. A P-value of <0.05 was considered statistically significant.

3. Results

Three hundred Jordanian men (150 infertile and 150 fertile men) were recruited from north and middle of Jordan to participate in the study. The infertile men included 77.3% oligozoospermic and 22.7% azoospermic cases. Demographic and semen parameters of all the subjects are shown in Table 2. Sperm mortality was different between fertile and infertile men. However, no differences between the two groups were found in the demographic characteristics, semen viscosity,

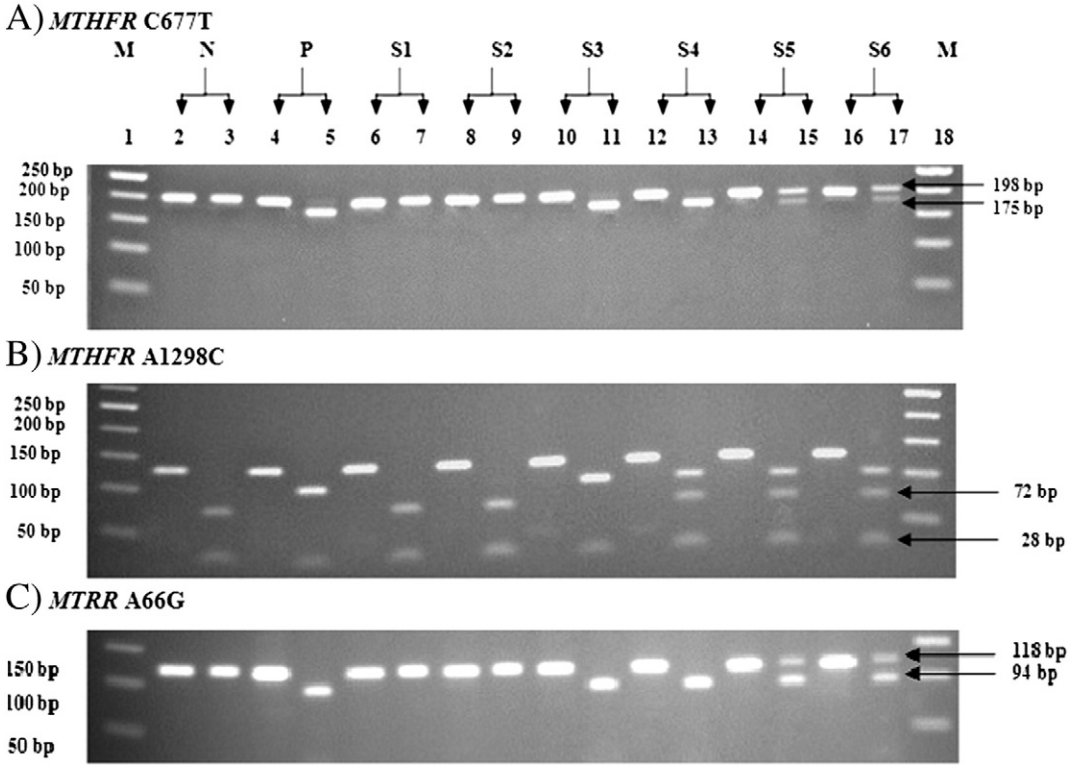

Fig. 1. Gel image showing PCR-RFLP pattern of the examined polymorphisms. Representative 3% agarose gel images of the examined polymorphisms. Lanes 1 and 18: 50 bp DNA ladder. N: negative control. P: homozygous positive control. Samples N, P and S1 through S6 represent results from this study, where the left lane of each sample is the undigested PCR product, while the right lanes are the digested products of the respective samples. A) *MTHFR* C677T PCR product by the restriction enzyme *Hinf* I. B) *MTHFR* A1298C PCR product by the restriction enzyme *Mbo* II. C) *MTRR* A66G PCR product by the restriction enzyme *Nsp* I. Arrows show digested fragments.