THE METHYLENETETRAHYDROFOLATE DEHYDROGENASE (MTHFD1) GENE G1958A POLYMORPHISM AND IDIOPATHIC MALE INFERTILITY IN A ROMANIAN POPULATION GROUP

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Abstract

Background. *The folate pathway consists of a complex chain of biochemical reactions essential for the cellular function. Within this pathway C1-THF synthase (C-1-tetrahydrofolate synthase) coded by the MTHFD1 gene is crucial for the conversion of homocysteine to methionine, as well as purine and pyrimidine synthesis. The MTHFD1 G1958A variant could alter the enzyme's normal activity which may further modify this metabolic pathway.*

Objectives. *To explore the possible association of the MTHFD1 G1958A polymorphism and idiopathic male infertility.*

Patients and Methods. *Sixty-six male Romanian patients with idiopathic infertility and 67 fertile Romanian men were genotyped for the MTHFD1 G1958A variant, using the polymerase chain reaction – restriction fragment length polymorphism technique (PCR-RFLP).*

Results. *The 1958A allele had a frequency of 52.27% in the male infertility group, and 45.52% in controls. The comparative analysis between patients and controls genotypes distribution revealed the 1958AA homozygous genotype was more frequent in the male infertility group than in controls (OR = 2.139, p-value = 0.07)*

Conclusions. *We have found that the variant homozygous genotype might represent a genetic risk factor for idiopathic infertility in our study groups.*

Keywords: methylenetetrahydrofolate dehydrogenase, idiopathic male infertility, polymorphism.

POLIMORFISMUL G1958A LA NIVELUL GENEI METILEN TETRAHIDROFOLAT DEHIDROGENAZA 1 (MTHFD1) ŞI INFERTILITATEA MASCULINĂ IDIOPATICĂ ÎNTR-UN GRUP POPULAŢIONAL DIN ROMÂNIA

Rezumat

Premize. *Calea metabolică a folaţilor rezidă într-un complex de reacţii esenţiale pentru funcţia celulei. În cadrul acestui lanţ, C1-THF sintetaza (C1-tetrahidrofolat sintetaza), codificată de gena MTHFD1, este crucială pentru conversia homocisteinei la metionină şi sinteza purinică şi pirimidinică. Polimorfismul MTHFD1 G1958A poate altera activitatea enzimatică normală şi modifica parametrii acestei căi metabolice.*

Obiective. *Analiza posibilei asocieri între polmorfismul MTHFD1 G1958A şi infertilitatea masculină idiopatică.*

Pacienţi şi metode. *Şaizeci şi şase de pacienţi români de sex masculin diagnosticaţi cu infertilitate idiopatică şi şaizeci şi şapte de bărbaţi fertili au fost genotipaţi pentru polimorfismul MTHFD1 G1958A, folosind tehnica PCR-RFLP (restriction fragment length polymorphism).*

Rezultate. *Alela 1958A a avut o frecvență de 52,27% în lotul de pacienți și 45,52% în lotul martor. Analiza comparativă a distribuției genotipurilor a arătat că genotipul homozigot 1958AA a fost mai frecvent întâlnit în lotul de pacienți, raportat la lotul martor.*

Concluzii. *Studiul demonstrează că genotipul homozigot 1958AA poate reprezenta un factor de risc pentru infertilitatea masculină idiopatică.*

Cuvinte cheie: metilentetrahidrofolat dehidrogenază, infertilitate masculină idiopatică, polimorfism.

Aims

Infertility represents a condition that has a major impact on the quality of life, and also important medical, social and familial implications in developed countries [1]. The male factor plays an important role in this pathology, being identified in almost half of the cases [2,3].

Genetic risk factors have been studied and incriminated in the etiology of male infertility, such as chromosomal abnormalities, AZF (azoospermia factor) microdeletions on the Y chromosome, or mutations of the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene [2]. In recent years one of the proposed factors in relationship to male infertility was the reduced levels of some nutrients: folates, zinc, vitamin B12, as well as hiperhomocysteinemia, which may be induced either by nutrition deficits or genetic causes [3,4,5]. Evidence was found that folates, homocysteine and cobalamine are important factors for spermatogenesis [6,7,8], for the stability of cellular membranes, or for the synthesis and maintenance of the DNA molecule [5,8].

It has been also shown that folate levels and folate dependent methylation can affect chromosomal segregation [2]. Within the genes coding for key enzymes in the metabolic pathway of folates and homocysteine, several polymorphisms have been shown to influence the production and function of the corresponding proteins [2,5,9]. One of these enzymes is C-1-tetrahydrofolate synthase (C1-THF synthase) encoded by the *MTHFD1* gene, enzyme with a triple function: 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase. Through the conversion of tetrahydrofolate, it contributes to the production of methionine from homocysteine and provides the one-carbon donors needed for nucleotides synthesis. Functional variants of *MTHFD1* gene may affect DNA synthesis with impact on cellular divisions. A polymorphism within the *MTHFD1* gene consisting in a nucleotide substitution G1958A has been studied in recent years as a possible variant, which may modify the activity of the C1-THF synthase enzyme and contribute to the risk of developing several pathological conditions [10].

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The purpose of this study was to analyze the distribution and the possible association of the *MTHFD1* G1958A polymorphism as a genetic risk factor in a group of male patients with infertility from the Romanian population.

Patients and methods

Our work was performed as a case-control observational study. A number of 133 men of Romanian origin were selected from patients referred to the Genetic Investigations II Laboratory, Emergency County Hospital for Adults Cluj, for investigations and genetic counseling. The subjects were divided into two groups, a group consisting of 66 men diagnosed with idiopathic infertility, after excluding other causes of infertility, such as varicocele, urogenital infections, endocrine dysfunctions, cryptorchidism, and other congenital morphological abnormalities. Also, chromosomal abnormalities and microdeletions of the Y chromosome were excluded by cytogenetic and molecular analyses. Out of the 66 patients, 54 of them were diagnosed with nonobstructive azoospermia, while the remaining were diagnosed with severe oligozoospermia. The control group comprised 66 fertile men who had at least one healthy child. Enrolment in the study was voluntary, according to the World Medical Association Declaration of Helsinki. Written and informed consent was obtained from all the participants of the study.

For genotyping the *MTHFD1* G1958A polymorphism, 2 ml of whole blood were withdrawn on EDTA from each patient. Genomic DNA was obtained using a commercial kit (Wizzard Genomic DNA Purification Kit, Promega, MA, USA), and then the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used as previously described [11], with minor modifications. Briefly, the PCR reaction was performed in a final volume of 25 µl containing: 12.5 µl PCR Mix–Taq DNA-polymerase 0.05 U/ml, $MgCl_2$ 4 mM, dNTP mix 0.4 mM each (Fermentas MBI, Vilnius, Lithuania); 1 µl BSA (bovine serum albumin, Fermentas MBI, Vilnius, Lithuania), 2 mg/ml solution, 8 pmoles of each primer (Eurogentec, Seraing, Belgium), approximately 100 ng genomic DNA, and nuclease-free water to the final volume. The reaction was set up on a MastercyclerGradient thermal cycler (Eppendorf, Hamburg, Germany). A

fragment of 330 bp was amplified and was then subjected to digestion at 37ºC overnight, using the restriction endonuclease MspI. Digested fragments were separated in ethidium bromide stained agarose electrophoresis gel (Metaphore Agarose, Lonza, Belgium).

In order to determine the possible association with male infertility the comparative analysis for the *MTHFD1* G1958A polymorphism distribution was performed for the whole group of infertile patients, as well as for the subgroups of patients with azoospermia (n=54), and severe oligozoospermia (n=12). In all three cases we considered the same control group composed of fertile men. Chi-square test was performed to test for 2 hypothetical risk models: the presence of at least one 1958A allele represents a risk factor in the heterozygous status as well as in the homozygous status; while in the second model we consider only the homozygous genotype 1958AA as a potential genetic risk factor for the studied pathology. The statistical analysis was performed using GraphPad InStat 3.10 and SPSS 16.0 for Windows softwares. Allele and genotype frequencies were compared using the χ^2 test. The approximate risk is presented as Odds Ratios with 95% Confidence Interval. Results were considered statistically significant at p-value < 0.05.

Results

Genotype and allelic distribution for the G1958A polymorphism are presented in table I.

By means of χ^2 test we tested for a possible deviation from the Hardy-Weinberg equilibrium (HWE), our results χ^2=0.1141, p=0,735 showing that the genotypes distribution in the studied population group is in accordance with HWE.

The results obtained for the comparative analysis show that the presence of at least one 1958A allele (dominant model) cannot be considered a risk factor for male infertility. In contrast to this observation, the homozygous genotype 1958AA (recessive model) could represent a genetic risk factor for male infertility in our study, although the association is only close to the level of statistical significance (OR 2.139; p=0.07). The data are presented in detail in table no. II. The same analysis performed by comparing the azoospermia subgroup with severe oligozoospermia subgroup yielded unsignificant differences (data not shown).

Discussion

To our knowledge, the present study represents the first attempt to analyze the possible association between the *MTHFD1* G1958A polymorphism as a genetic risk factor for male infertility in Romanian population. There are only two other studies performed on the Romanian population: one concerning the possible association with spontaneous recurrent abortions [12] and a second one which investigated the association with Down syndrome [13], both of them showing that this polymorphism would not be a risk factor for the studied pathologies. The observed allelic frequency for the 1958A allele in these studies (40-45%) is slightly lower than our observed frequency (45-52%). Furthermore, in a study concerning the frequency of 51 polymorphisms associated with different forms of pathology, the estimated global frequency of this polymorphism ranges from 24% to 57%, with the highest value being found in white Hispanics and the lowest in Afro-Americans, while in Caucasians the cited frequency is 45% [14]. The frequency of homozigous 1958AA genotype in our patients group compared to controls, might explain the higher frequency of the 1958A allele than in the above mentioned studies. Three polymorphisms of genes which are known to play a critical role in the DNA synthesis and remethylation (methylenetetrahydrofolate reductase - *MTHFR*, methionine synthase - *MS* and MS reductase - *MTRR*) have been shown to be independently associated with male

Table I. Allele and genotype frequencies for *MTHFD1* G1958A in infertile males and controls.

	Alleles frequencies, n (%)		Genotypes frequencies, n (%)		
	G allele (common)	A allele (variant)	1958GG	1958GA	1958AA
All infertility cases (n=66)	63 (47.72)	69 (52.27)	18 (27.27)	27 (40.90)	21 (31.81)
Azoospermia (n=54)	55 (50.92)	53 (49.07)	17 (31.48)	21 (38.88)	16 (29.62)
Severe oligozoospermia (n=12)	8 (33.33)	16 (66.67)	1 (8.33)	6 (50.00)	5 (41.66)
Controls (n= 67)	73 (54.47)	61 (45.52)	18 (26.86)	37 (55.22)	12 (17.91)

Table II. Genotypes distribution analysis as risk factor for male infertility group compared to controls.

Model	Pathology	Odds ratio	p value	95% CI
MTHFD1 G1958A	All infertility cases	0.799	1.00	0.455 – 2.106
GA+AA genotypes vs.	Azoospermia subgroup	0.979	0.687	0.363 – 1.759
GG genotype	Severe oligozoospermia subgroup	4.041	0.274	0.486 – 33.588
MTHFD1 G1958A	All infertility cases	2.139	0.07	0.950 - 4.815
AA genotype vs.	Azoospermia subgroup	1.930	0.136	0.820 – 4.539
GA+GG genotypes	Severe oligozoospermia subgroup	3.274	0.119	0.886 – 12.095

infertility [2,15,16]. The relation between male infertility and polymorphisms of other important genes of the folate-homocysteine pathway, such as cystathionine beta-synthase (*CBS*) or the gene encoding the reduced folate carrier (*SLC19A1*) could not been demonstrated [17,18]. The variant *MTHFD1* G1958A was identified as a risk factor for neural tube defects in Irish and Italian populations [11,19,20], with intrauterine growth restriction, gastric cancer, and schizophrenia [21]. It has been presumed recently that the polymorphism does not affect the synthase function of the protein; rather it decreases the thermostability of the enzyme, thus reducing the synthesis of purines [10]. The same polymorphism has been proposed to reduce the synthesis of 5,10-methylenetetrahydrofolate, which may subsequently affect the conversion of homocysteine to methionine and impair the remethylation cycle [11]. This metabolism is involved in the methylation of nucleic acids, and in DNA synthesis and repair, essential processes for normal spermatogenesis. At least two mechanisms could explain alteration of the spermatogenesis through impaired folate pathway: DNA hypomethylation leading to altered gene expression and uracil missincorporation during DNA synthesis, leading to errors in DNA repair, strand breakage and chromosomal anomalies [9].

Conclusions

The present study brings new data concerning the possible impact of genetic polymorphisms as risk factors for male infertility, and also offers insights on genetic epidemiological data in the Romanian population, data overlooked to date. The present study has some limitations, such as the impossibility to determine the plasmatic levels of folic acid and homocysteine, as well as the relatively small number of cases, especially in the severe oligozoospermia subgroup. However the data we obtained show that the homozygous variant genotype of the *MTHFD1* G1958A polymorphism might be associated with male infertility. It is also important to take into account that this is a multifactorial type of pathology and we cannot ignore the combined influence of other genetic or environmental risk factors which remain to be clarified by the future studies.

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