DISTURBANCES OF FOLATE METABOLISM IN MEN WITH INFERTILITY

Article history:
Submitted 18 March 2019
Accepted 11 May 2019

Galina Myandina, Nina Kulchenko*, Hasan Alhejoj, Ekaterina Tarasenko

Peoples' Friendship University of Russia (RUDN University), Moscow, Russia

**Corresponding Author: kle_kni@mail.ru*

ABSTRACT — Aim. To study the association between the male infertility and the -1298A/C (rs1801131) polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene with male infertility in the Moscow region.
Methods. Study group of infertile men (n=70) includes 26 non-obstructive azoospermic patients, 23 astenospermic and 21 patients with teratospermia and 68 healthy controls with normal sperm parameters who had one child and more. Genotyping was performed by generated amplicons from melting curve analysis after real time PCR.
Results. We did not receive significant differences in the frequencies of MTHFR genotypes (A1298C) in infertile and fertile men. In the subgroup of men with pathospermia, carriers of the minor allele 1298C (genotype AC + CC) were found with a frequency of 61%, in the group of fertile men the frequency of carriers of the allele 1298C was 53% ($\chi^2 = 1.01$; $p = 0.31$). However, the frequency of the minor allele 1298C of the MTHFR gene in the subgroup of men with asthenozoospermia was 50%, in the subgroup with teratozoospermia it was 33% and in the subgroup of men with azoospermia — 39% ($\chi^2 = 8.67$; $p = 0.003$).
Conclusions. Genetic polymorphism of the locus A1298C (rs 1801131) of folate metabolism gene MTHFR is associated with risk of violations of motility of sperm in men of the Moscow region of Russia.

KEYWORDS — Folate metabolism, male infertility, asthenozoospermia, gene polymorphism, methylenetetrahydrofolate reductase (MTHFR) gene

INTRODUCTION

The frequency of male infertility reached 17.2% in Russia [1]. Today, all authors tend to assume that the most frequent endogenous cause of male infertility is oxidative stress [1, 3, 7]. Oxidative stress occurs in 30–80% of cases in men with infertility [4]. The development of scientific and technological progress has led to environmental degradation, the growth of chronic diseases, which leads to the accumulation of reactive oxygen species (ROS). In a healthy man, the level of ROS and antioxidant system are balanced [1, 4]. Folate deficiency leads to an imbalance of the methyl groups involved in the synthesis and methylation of DNA, and increased oxidative stress [3, 6] According to Kurzawski M. et al. polymorphism of the folate metabolism gene methylenetetrahydrofolate reductase — MTHFR 1298A> C (rs1801131) is associated with a decrease in enzyme activity by 30% [2]. The association of polymorphisms of the MTHFR 1298A> C (rs1801131) and patospermia was not studied in the Moscow region.

Aim

To determine the association of polymorphic locus A1298C (rs 1801131) of the folate metabolism gene MTHFR with the risk of male infertility in the Moscow region.

METHODS

The study included 70 infertile patients (according to the conclusions of the spermogram, WHO 2010) — 1 group of patients, and 68 fertile men (with one or more children) — the control group. All men were aged 30±6 years, and lived in the Moscow region, agreed to the study. All patients underwent standard clinical examination, determination of karyotype and presence of deletions of AZF-region of Y-chromosome, mutations of CFTR gene.

To study the association of MTHFR A1298C (rs 1801131) gene polymorphism, we isolated DNA from peripheral blood leukocyte mass. Polymorphism of folate exchange genes were detected by polymerase chain reaction (PCR) in real time (Real-Time-PCR). For real time PCR used ready-made kits to identify polymorphisms in genes of folate metabolism (the company "Synthol", Russia). Genotyping was performed by generated amplicons from melting curve analysis after real time PCR on the basis of Tag Man method of probes with respect to fluorescence (RFU) of each probe. The study was performed at laboratory of Biology and General genetics department of the Peoples ' Friendship University of Russia. The genotypes of homozygotes for the reference allele (AA), heterozygous carriers with genotype (AC) and homozygotes for the minor allele of the (CC) were detected.

The frequency of alleles of the polymorphisms studied in the groups of patients and fertile men was compared using the χ^2 criterion. The significance of the differences was assessed by the nonparametric criterion

"Obs/Exp", the differences were considered significant at p<0.05. The data obtained were subjected to treatment with the use of STATISTICA 6.0

RESULTS

The most number of patients of the first group were with impaired sperm motility (Fig.1). The percentage of progressive motile sperm of category a+b was significantly lower in men with pathospermia compared to the control group: 18.6+5.7% versus 29.6+2.8% (p<0.05). The percentage of normal morphological forms of sperm also significantly differed between the first and second group of men: 2.8+2.3% and 21.7+9.1% respectively (p<0.05).

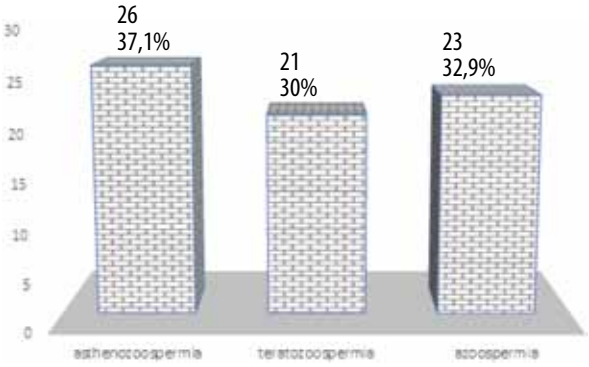

Fig. 1. *Distribution of patients of the first group in terms of sperm*

All the men we examined had normal karyotype, no mutations in the CFTR gene and microdeletions of AZF locus in the Y chromosome. It was excluded the infertility of their wives.

We compared the allele frequency distribution of the studied MTHFR gene polymorphism in both groups of men: infertile and fertile. For A1298C polymorphism of MTHFR gene, the frequencies of A/A, A/C, C/C genotypes were 38.57%, 42.85%, 18.57% among infertile patients and 47.05%, 42.64%, 10.29% in the group of fertile men (χ^2 = 2.20; p=0.33).

In the subgroup of men with pathospermia, carriers of minor allele 1298C (genotype AC+CC) were detected with a frequency of 61%, in the group of fertile men the frequency of allele carriers 1298C was 53% (χ^2 = 1.01; p=0.31).

Since we have not received significant differences in the genotypes frequencies of MTHFR (A1298C) in infertile and fertile men, we decided to conduct a comparative analysis of genotypes frequencies of the locus A1298C (rs 1801131) of folate metabolism gene MTHFR in subgroups of patients with different forms of pathospermia (Table 1). The frequencies of heterozygous carries of minor allele 1298C of the MTHFR gene (genotype AC) in the subgroup of men with asthenozoospermia was 50%, in the subgroup with teratospermia was 33% and in the subgroup of men with azoospermia — 39% (χ^2=8.67; p=0.003).

Table 1. *Distribution of the frequencies of polymorphic locus A1298C (rs 1801131) of the MTHFR gene in infertile men with different types of pathospermia*

Genotype / Sperm values	Homozygoues for the reference allele (AA)	Heterozygous carriers (AC)	Homozygoues for the minor allele (CC)
Asthenozoospermia (n(%)	6(23%)	13(50%)*	7(27%)*
Teratozoospermia (n(%)	10(48%)	7(33%)	4(19%)
Azoospermia (n(%)	11(48%)	9(39%)	3(13%)

** p<0.05 in carriers of minor allele with impaired sperm motility compared to other sperm parameters*

DISCUSSION

Folates are nutrients that are involved in many metabolic processes in the human body. In men, folates are necessary for the synthesis of sperm DNA and are involved in maintaining the integrity of the genome [6]. Folate deficiency can worsen the DNA methylation, lead to the accumulation of homocysteine, which further leads to excessive oxidative stress and disruption of DNA repair [3].

To date, published data on the association of the polymorphism of the folate metabolism gene MTHFR A1298C (rs1801131) with the risk of male infertility are inconclusive. This association has not been identified for some areas of China [5]. In some European populations there were not detected any significant differences between fertile and infertile men according to MTHFR gene polymorphisms [2].

It was shown by Shen O. et al. that MTHFR A1298C was associated with a significant increased risk of azoospermia when comparing homozygotes (CC vs. AA) (OR = 1.66) [6]. The authors believe that the C allele of MTHFR is a genetic risk factor for male infertility [6]. The results of our study demonstrate the association of folate gene polymorphism MTHFR A1298C with different forms of pathospermia (p=0.003) for infertile men. Consequently, it can be assumed that the A1298C polymorphism of the MTHFR gene may be involved in the etiology of male infertility in patients of the Moscow region. Probably, genetic variations in the genes of enzymes associated with folates metabolism may be associated with male infertility.

CONCLUSIONS

Polymorphic locus A1298C (rs 1801131) of the MTHFR gene for folate metabolism is associated with the risk of asthenozoospermia in men in the Moscow region of Russia. We believe that in infertile men, especially with impaired sperm motility, it is necessary to carry out molecular genetic measures to identify the carriers of the allele 1298C of the MTHFR gene in order to choose a personal therapeutic tactics in overcoming the factor of infertility.

REFERENCES

1. **Kulchenko N.G.** Oxidative stress in the development of non-obstructive azoospermia. Difficult patient. 2017;15(4-5):44–46
2. **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. Genetics and molecular biology. 2015; 38(1): 42–47, doi: 10.1590/S1415-475738120140179
3. **Montjean D., Benkhalifa M., Dessolle L., Cohen-Bacrie P., Belloc S., Siffroi J. P.** Polymorphisms in MTHFR and MTRR genes associated with blood plasma homocysteine concentration and sperm counts. Fertility and Sterility. 2011; 95: 635–640. DOI: 10.1016/j.fertnstert.2010.08.054
4. **Myandina G. I., Kulchenko N. G., Alhejoj H.** Polimorfism G-105A SEPS1 Gene and Mens's Infertility. Medical News of North Caucasus. 2018; 13(3): 488-490. https://doi.org/10.14300/mnnc.2018.13085
5. **Ni W., Li H., 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 Chinese population. J Assist Reprod Genet. 2015; 32(3): 369-374, Doi 10.1007/s10815-014-0423-9
6. **Shen O, Liu R, Wu W, et al.** Association of the methylenetetrahydrofolate reductase gene A1298C polymorphism with male infertility: a meta-analysis. Ann Hum Genet, 2012; 76(1): 25–32, https://doi.org/10.1007/s11596-017-1709-9
7. **Vinogradov I.V., Zhivulko A.R., Vinogradova L.M., Korolev S.V.** Docosahexaenoic acid in the treatment of male infertility. Andrology and Genital Surgery. 2018;19(4):21–27. https://doi.org/10.17650/2070-9781-2018-19-4-21-27