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NEURAL TUBE DEFECTS AND POLYMORPHISM OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE IN KAZAKH POPULATION

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The mutations of MTHFR gene in various populations are considered as risk factors for neural tube defects. The frequencies of MTHFR genotypes among the mothers of NTD cases were: CCaa - 15,4%, CCac - 15.4%, CCcc - 10%, CTaa - 16.9%, CTac - 30.8%, CTcc - 6.9%, TTaa - 3.8%, TTac - 0.8%. The frequencies of this genotypes among controls were: CCaa - 29%, CCac - 21%, CCcc - 6%, CTaa - 30%, CTac - 13%, CTcc - 1%, TTaa - 1%. Thus, the frequencies of CTac, CTcc, TTaa, TTac genotypes of C677T and A1298C polymorphisms of MTHFR in the basic group authentically exceed similar parameters in the group of healthy control ($p<0.05$).

The research indicates on presence of clinical and diagnostic significance of the investigated polymorphisms of the MTHFR gene in the development of NTDs. Ascertainment of the etiologic aspects of fetal NTDs in the Kazakh population makes it possible to develop preventive measures and optimization of medical genetic counseling in order to reduce fetal NTDs in the population.

Keywords: Methylenetetrahydrofolate reductase, neural tube defects, polymorphism.

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Introduction

One of the important tasks of medical genetics is the study of the etiologic basis of hereditary and congenital disorders. Determination of genetic factors in the development of hereditary diseases allows working out an effective prevention, which, along with early diagnosis can help to reduce the incidence of congenital and hereditary anomalies in the population.

According to Watson and Sellers (2005) one of the most severe and frequent congenital defects (CDF), contributing a significant endowment to indicators of perinatal morbidity, disability and death are fetal neural tube defects (NTDs).

According to Busby et al. (2005) functioning of various monitoring systems of CDF shows that frequency of the NTD in the world varies from 0.2 to 10 per 1000 newborns per year. In Kazakhstan, according to the National genetic register (NGR) of congenital defects in infants between 1998 and 2010, there were recorded about 1486 cases of newborns with various NTDs. The average frequency of NTDs in the country amounted to 0.7 per 1000 births. In this case, NTDs annually ranks first in the structure of stillbirth and third place in the perinatal mortality from CDF. Furthermore, NTDs contribute greatly to the rates of infant morbidity and disability, and infant mortality from associated diseases or postoperative complications.

The majority of NTDs in Kazakhstan are diagnosed during pregnancy by ultrasonic diagnosis. On average, up to 80-85% of SMG and up to 100% of anencephaly is detected before 20 weeks of pregnancy. As a result of successful prenatal diagnosis, 1113 pregnancies was interrupted due to NTDs in the fetus in Kazakhstan since 2007, which accounted for approximately 30% in the structure of reasons of induced pregnancies on genetic indications. The frequency of newborns with this pathology has decreased from 0.7% in 2000 to 0.4% in 2010. Thus, as one of the main reasons of induced pregnancies

on genetic indications, NTDs contribute significantly to the violation of women' health of reproductive age.

Thus, the early development of NTDs in the prenatal period (28 days of gestation), severe abnormalities, high rate of fetal and newborn dictate the necessity of search for etiologic factors of NTDs occurrence and the development of preventive measures in periconceptional period. The causes of occurrence of the majority of NDTs have not been determined yet.

According to Bochkov (1997) 10% of the causes of NTDs are monogenic or chromosomal abnormalities, the most frequent of which - trisomies 18, 13 and 21, syndromes of Meckel-Gruber, Walker-Warburg, cryptophthalmos, Roberts and etc.. According to numerous studies (Brender et al., 2006; Edwards et al., 1995; Felkner et al., 2003), a large number of external factors can affect the development of NTDs: ethnic and geographical, demographic and reproductive features of population, social factors, medication intake, presence of mother's extragenital pathology, peculiarities of the diet, etc. Volcik et al. (2000) indicate that 80% of NTDs have multifactorial nature, i.e. appear in the presence of genetic predisposition and an additional external factor and as a major genetic factor in the development of NTDs are considered Methylenetetrahydrofolate reductase gene mutation (MTHFR), which is a key gene of folate metabolism.

In Botto and Yang (2000) there were studied several types of mutations in the MTHFR gene and two polymorphisms were recognized as the most significant of them. The mutation caused the replacement of cytosine to thymine at position 677 related to 4 exon and denoted as C677T, leads to a decrease of MTHFR enzyme function from normal to 35%. The second polymorphism is caused by replacement of adenine to cytosine at position 1298, designated as A1298S and leads to the formation of an enzyme with activity decreased to 60% (Botto and Yang, 2000). Biochemical defects with low MTHFR enzyme activity due to the presence of mutations in the MTHFR gene are characterized by the development of hyperhomocysteinemia and a violation of the synthesis of nucleic acids and proteins due to insufficient absorption of folic acid. These processes contribute to disruption of fetal neural tube imperforation in the early stages of embryogenesis and development of NTDs (Botto and Yang, 2000).

According to Botto and Yang (2000), Sadewa et al. (2002), Volcik et al. (2000), polymorphism of MTHFR gene and its genetic contribution to the etiology of NTDs is studied in many European and some Asian populations. The obtained results are controversial, i.e. the identification of etiological aspects of NTDs in various ethnic populations, including the Kazakh, remains today an unresolved problem.

Significant interpopulation and intrapopulation differences in the frequency of NTDs and the role of C677T and A1298S mutations carriage of MTHFR gene makes it necessary to study the epidemiological features and molecular genetics aspects of the development of NTDs in the populations of the Republic of Kazakhstan.

In order to improve medical genetic counseling (MGC) for NTD and effective prediction of pathology leading to selection by environmental and genetic risk factors, thus allows to form risk groups for NTDs, develop effective measures of primary prevention and reduce the incidence of this disease in the population of Kazakhstan.

The aim of the study was to determine the clinical and diagnostic significance of polymorphisms C677T and A1298C of MTHFR gene in the development of NTDs in the Kazakh population.

Material and methods

During the research were used the following materials: the DNA samples extracted from peripheral blood of 130 women who had a history of cases of children born with various NTDs. As a control material were used DNA samples of 100 healthy Kazakh women with 2 or more healthy children.

During the research were used following methods: molecular genetic analysis, including extraction of DNA from 5 ml peripheral blood samples, conducting a polymerase chain reaction (PCR) using specific oligonucleotide primers and the restriction fragment length polymorphism analysis (RFLP) using the enzyme Hinf I.

Statistical analysis was performed by standard methods of processing the results of the calculation of χ^2 test and the coefficient of relative risk odds ratio (OR).

Results and discussion

To determine the clinical and diagnostic significance of genetic polymorphism C677T of the MTHFR gene were determined by the frequency of alleles and genotypes for this polymorphism in the main and control groups. The results are presented in Table 1.

Due to the materials in Table 1, in the study group was significantly less frequent favorable homozygous genotype (CC) compared with healthy control (χ^2. = 6.5; $p < 0.05$). Frequency of heterozygotes (CT) and homozygotes for the T allele (TT) in the intervention group was significantly higher than in the control group ($p < 0.05$). Significant differences in the frequency of alleles T and C in the studied groups were not revealed (χ^2. = 2.7; $p > 0.05$).

TABLE 1. FREQUENCY OF GENOTYPES AND ALLELES OF MTHFR GENE DUE TO C677T POLYMORPHISM IN THE GROUPS STUDIED (%)

Genotypes, alleles	Basic grope		Control grope	
	n	%± m	n	%± m
CC	53	40.8±4.3	56	56.0±4.9
CT	71	54.6±4.4	43	43.0±4.9
TT	6	4.6±1.8	1	1.0±0.79
Total	130	100.0	100	100.0
C	177	68.1±2.9	155	77.5±3.0
T	83	31.9±2.9	45	22.5±2.9
Total	260	100.0	200	100.0

The results of the analysis of A1298C polymorphism of the MTHFR gene have revealed the following frequencies of genotypes and alleles: homozygous variant on the normal allele, aa - 33.8 ± 4.2, heterozygous genotype ac - 52.3 ±4.4, homozygous genotype for the mutant allele cc - 13.8± 3. Allele frequencies were as follows: a - 60±3, c - 40±3.

When comparing the frequencies with similar data of the control group was found a significant contribution to the carriage of adverse alleles c, and heterozygous (ac) and homozygous (cc) genotypes in the occurrence of NTDs in the fetus of the Kazakh population ($\chi^2 = 13.2$; $p < 0.005$ and $\chi^2 = 6.1$, $p < 0.05$).

The distribution of polymorphic variants of MTHFR gene in both polymorphisms revealed that the most favorable genotype, occurring significantly more frequently in the control group, was genotype - CCaa ($\chi^2 = 27.2$; $p < 0.0005$) (Table 2).

Frequency of most adverse genotypes TTaa, TTac and CTcc was significantly higher than the one of healthy control data. In this case, it was noted that the options TTac and CTcc were found only in the main group. The research revealed the presence of significantly higher frequency of adverse genotype due heterozygous for both alleles (CTac) in the intervention group compared with controls.

Thus, the first time determined the frequency of polymorphic variants of MTHFR gene in a group of mothers with NTD fetus and in healthy controls. The comparative analysis of molecular genetic studies indicates the presence of the genetic contribution of the adverse genotypes and alleles of the MTHFR gene in the etiology of NTDs of Kazakh women.

Analysis of the carrier frequency of polymorphic variants of MTHFR gene of children with NTDs and their parents allowed us to determine the coefficient of odds ratio (OR), calculated as the relative risk for NTDs in carriers of a particular genotype. OR - factor that determines the degree of risk of disease based on the genetic origin, used in population genetics. The higher the value of OR, the more is the unfavorable prognosis of this disease.

TABLE 2. FREQUENCIES OF GENOTYPES OF THE MTHFR GENE ON THE TWO POLYMORPHISMS C677T AND A1298C OF KAZAKH WOMEN IN THE STUDY AND CONTROL GROUPS (%)

Gaplotypes	Basic grope n=130		Control grope n=100	
	n	%± m	n	%± m
CCaa	20	15.4±3.2	29	29± 4.5
CCac	20	15.4±3.2	21	21± 4.1
CCcc	13	10±2.6	6	6± 2.4
CTaa	22	16.9±3.3	30	30± 4.6
CTac	40	30.8±4	13	13± 3.4
CTcc	9	6.9±2.2	-	-
TTaa	5	3.8±1.7	1	1± 0.9
Ttac	1	0.8±0.2	-	-
TTcc	-	-	-	-

Analysis has shown that the genotype of TTaa, conditioned by homozygosity for the T allele of C677T polymorphism and homozygosity for a allele of a polymorphism in A1298C, increases the risk of NTDs in 3.9 times.

The least favorable option for the haplotype in which the degree of relative risk of developing this disease increases 15.9 times, was genotype CTcc due to heterozygosity for C677T polymorphism and homozygous for c allele of a polymorphism in A1298C. Carrier state of genotype CTac also increases the risk of NTDs in 3.5 times, genotype TTac - in 2.6 times. The results for the assessment of the risk of NTDs in the Kazakh population for carriers of a particular genotype will determine the likelihood of developing disease and to work out the folate prophylaxis before pregnancy.

To clarify the role of unfavorable genotypes of the MTHFR gene in the development of various forms of NTD was hold the correlation analysis, which establishes the relationship of genotype with the severity level of the localization of neural tube defect. Genotypic variants of the mothers were divided by severity into ascending order. NTDs are in terms of localization, ranging from less severe spina bifida of sacrococcygeal to anencephaly. The analysis showed that carriers of genotypes CTcc and TTac is most common in high-DNT localization - anencephaly and spina bifida of the cervical and thoracic, Spearman correlation coefficient $r = 0.5$ ($p = 0.003$).

Thus, it was found that the most unfavorable genotypes of the MTHFR gene are characteristic of NTD of a high level, which by the nature of lesions are more severe and in most cases, lethal.

The molecular genetics research leads to the conclusion about the presence of clinical and diagnostic significance of the investigated polymorphisms of the MTHFR gene in the development of NTDs. Ascertainment of the etiologic aspects of fetal NTDs in the Kazakh population makes it possible to develop preventive measures and optimization of medical genetic counseling in order to reduce fetal NTDs in the population.

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