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Analysis of Polymorphism of Allelic Variants of the ADRB3 Gene for the Risk of Miscarriage

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Abstract The study involved pregnant women aged 20 to 40 years who were under the supervision of an obstetrician-gynecologist on an outpatient and inpatient basis. All pregnant women underwent clinical, functional, molecular genetic studies. The results of molecular genetic studies have shown that the unfavorable variant allele "C" of the rs4994 polymorphism of the ADRB3 gene, leading to the replacement of T with C, is possibly related to the development of miscarriage in pregnant women. It was found that the risk of developing MC anomalies in pregnant women in the presence of a variant allele of polymorphism in the genome increased by 1.7 times (OR=1.8).

Keywords Miscarriage, Prediction, ADRB3 gene, Genotypes of the T/C gene, C/C of the ADRB3 gene

1. Introduction

According to statistics, the detection rate of miscarriage (MC) ranges from 9.6% to 25.2% of cases. Clinical observations indicate that the highest frequency of MC is observed in the first trimester 51.2-70.1% of cases, in the second trimester - 17.8-21.1%, and in the third trimester - 6.7 to 30.1%. The leading role in the etiology of early spontaneous miscarriages is occupied by numerical chromosomal aberrations [1,2,5,7]. MC in the III trimester is the cause of more than 50% of stillbirths, 70-80% of early neonatal mortality, 60-70% of infant mortality [2,6,9].

In the structure of MC habitual miscarriage is about 24.9% of cases. According to domestic authors, early habitual miscarriage (HMC) is the presence of two or more spontaneous miscarriages before 16 weeks, some foreign clinicians still refer to this term as a three-time spontaneous abortion before the 12th week [3,5,6,10,13].

Recently, depending on the obstetric history, two forms of MC are distinguished: primary - when all pregnancies ended in spontaneous miscarriages and secondary - when in the anamnesis, along with miscarriages, there were and / or medical abortions, childbirth, ectopic pregnancy.

One of the leading causes of MC in early terms is considered to be a genetic factor. Over the past 50 years, the concept of "genetic causes of EE" included only the presence of chromosomal aberrations (quantitative and / or structural abnormalities in the chromosome set) both in spouses with a history of miscarriages and in abortions [4,7,8,11,12]. At the present stage, new high-tech molecular diagnostic methods are widely used, and the concept of "genetics of miscarriage" has expanded its boundaries. MC can be caused by chromosomal abnormalities, gene mutations and the presence of a hereditary predisposition.

Numerous scientific studies have been devoted to the genetic aspects of MC, where a significant assessment is given to genes, enzymes of xenobiotics of the first and second phases, folate metabolism (MTRR); coagulation factors, growth factors (UECF). However, in the literature there is no study of the ADRB3 gene responsible for the activity of the sympathetic nervous system, and is also involved in the predetermination of lipolysis (destruction of fat cells, adipocytes) and thermoregulation [4,7,8,11].

The purpose of the research. To study allelic variants and associations of ADRB3 gene genotype polymorphisms with the risk of miscarriage.

2. Material and Research Methods

We examined 79 pregnant women aged 20 to 40 who were under the supervision of an obstetrician-gynecologist on an outpatient and inpatient basis. All pregnant women underwent clinical, functional, molecular genetic studies. The control group consisted of 35 pregnant women with a physiological course of pregnancy.

As part of the study, there was a genetic analysis of biological blood samples from 79 female patients presented to determine the genotypic polymorphism of the ADRB3 gene, consisting of T/C (rs4994) alleles. DNA/RNA isolation from all biological blood samples was performed using the Ribo-prep kit (Interlabservis, Russia).

To identify the polymorphism of the genotype consisting of T/C alleles (rs4994 of the ADRB3 gene), allele-specific primers from the manufacturer were selected from DNA samples.

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For genotyping of DNA samples by polymerase chain reaction (PCR), 79 DNA samples were studied. For this, the 96-cell automated amplifier "Applied Biosystems Veriti" was optimized according to the following program: initial denaturation once at 180 sec 94℃, 94℃ - 10 sec, 64℃ - 10 sec, 72℃ - 20 sec in the program we did these indicated steps 40 times for the polymerase chain reaction to occur. Statistical analysis of the results was carried out using the statistical software package "OpenEpi 2009, Version 2.3".

3. Results

Molecular genetic analysis of the studied gene was carried out taking into account allelic variants and genotypes of the ADRB3 gene. The control group consisted of women without MC (35 patients). (Table 1)

The results of the study of allelic variants of the ADRB3 gene in the control group of patients without MC showed the same frequency of detection of both a favorable allele T and an unfavorable allelic variant C of the ADRB3 gene was 4.2% of cases (35/70). Analysis of the frequency of occurrence of 50% of cases, respectively. Whereas in the main group of pregnant women with MC, the frequency of a favorable allelic T variant was 39.7% (35/88) of cases ($\chi2$=1.65 p<0.2; OR=0.66; 95% CI 0.35-1, 24), while the unfavorable allele C was 60.2% (53/88), respectively. The mutant allele C in the main group of pregnant women with MC was 1.2 times higher than in the control group. ($\chi2$=1.65 p<0.2; OR=1.51; 95%CI 0.8-2.85).

The analysis of the data obtained indicates the absence of an association between the mutant allele "C" (rs4994) of the ADRB3 gene and miscarriage, despite the high odds ratio (OR= 1.5).

An analysis of the association of ADRB3 gene genotypes in the control group revealed the identification of favorable T/T genotypes in 28.6% (10/35), while in the main group this genotype was 11.4% (5/44), which is 2, 5 times lower than the control group ($\chi2$=3.86 p<0.15; OR=0.32; 95% CI 0.10-1.05). Whereas the heterozygous genotype T/C of the ADRB3 gene in the control group was determined in 42.8% of cases (15/35), and in the main group of pregnant women with MC it was 56.8% (25/44), which is 1.3 times higher than the parameters of the control healthy patients without MC. ($\chi2$=3.86 p<0.15; OR=1.75; 95%CI 0.72-4.3). And the unfavorable m genotype C/C of the ADRB3 gene in the control group was determined in 28.6% of cases (10/35). and in the main group it was 31.8% (14/44), which is 1.1 times higher than those in the control group ($\chi2$=3.86 p<0.15; OR=1.17; 95% CI 0.44- 3.08).

Taking into account the fact that in the main group there was a significant detectability of the association of polymorphism of unfavorable genotypes 1.3 times more than that in the control group, the data obtained may indicate that the carriage of the heterozygous genotype of the ADRB3 gene polymorphism may be a predisposition factor to the development of this pathology, increasing its risk by 1.7 times (OR=1.75) (Table 2).

Comparison of the obtained results with the clinical course of pregnancy showed that in 21 patients of the main group in the identified mutant allelic variants C of the ADRB3 gene, miscarriage was noted in the first trimester, which accounted for 47.7% of cases, and in 10 pregnant women - in the second trimester, which was 22.7% and in the III - trimester - in 8 (18.2%) cases, respectively. Whereas in the group of pregnant women with favorable T alleles of the ADRB3 gene, HB was observed in 2 in the I trimester, which amounted to 4.5% and in 3 in the II trimester, which amounted to 6.8% of cases, respectively.

Table 1. Distribution frequency of allelic variants and T/C ADRB3 gene polymorphism in pregnant women with MC and control healthy group of pregnant women

№	Group	Allele frequency				Distribution frequency of genotypes					
		T		C		T/T		T/C		C /C	
		n	%	n	%	n	%	n	%	n	%
1	Main group n=44(88)	35	39.7	53	60.2	5	11.4	25	56.8*	14	31.8
2	Control group n=35(70)	35	50	35	50	10	28.6	15	42.8	10	28.6

N - the number of examined patients; *n - number of studied alleles;
* - significance indicator in relation to the control group (P<0.05)

Table 2. Differences in the frequency of occurrence of alleles and genotypes of the T/C (rs4994) polymorphism of the ADRB3 gene in the main and control groups of pregnant women

Alleles and genotypes	Number of examined alleles and genotypes		Statistical difference
	Main group	Control group	
Allele T	35	35	χ^2=1,65 p<0,2; OR=1,51; 95%CI 0,80-2,85
Allele C	53	35	
Genotype T/T	5	10	χ^2=3,86 p<0,15; OR=0,32; 95%CI 0,10-1,05
Genotype T/S	25	15	χ^2=3,86 p<0,15; OR=1,75; 95%CI 0,72-4,3)
Genotype C / C	14	10	χ^2=3,86 p<0,15; OR=1,17; 95%CI 0,44-3,08

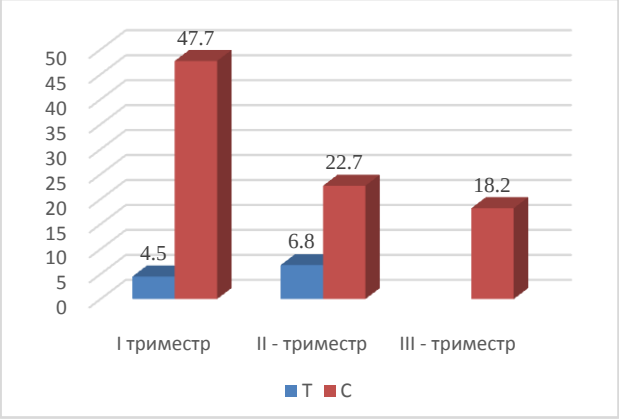

Figure 1. The indicator of the frequency of miscarriage, taking into account allelic variants of the ADRB3 gene (%)

Thus, the results of molecular genetic studies have shown that the unfavorable variant allele "C" of the rs4994 polymorphism of the ADRB3 gene, which leads to the replacement of T with Cr at position 4994 of the amino acid sequence, may be associated with the development of miscarriage in pregnant women. It was found that the risk of developing MC in pregnant women in the presence of a variant allele of polymorphism in the genome increased by 1.7 times (OR=1.8).

The result obtained also indicates that the heterozygous T/C genotype of the ADRB3 rs4994 polymorphism is a genetic determinant, which is a predisposition factor for the development of this pathology, increasing its risk by 1.7 times (OR=1.8). The data obtained require close attention from obstetrician-gynecologists.

According to the literature data, the population frequency of occurrence of various allelic variants and genotypes of polymorphic genes can be variable, as it is influenced by various dynamic factors involved in creating the genetic structure of a population. At the same time, it is important to assess the expected and observed frequency of genotypes of the studied polymorphic genes, potentially associated with the development and pathogenesis of diseases, which can be determined in accordance with the distribution of frequencies according to the Hardy-Weinberg equilibrium (HW).

Table 3. Expected and observed distribution frequency of genotypes according to HWE of the T/Cr rs4994 polymorphism of the ADRB3 gene in the main group of pregnant women with MC

Genotypes	Frequency of genotypes		χ^2	P
	observed	expected		
T/T	11.4	46.8	0.158	0.37
T/C	56.8	43.2	0.479	
C/C	31.8	9.9	0.363	
Total	100.00	100.00	0.0	

As follows from Table 3, distribution frequency of genotypes according to HWE of the T/C (rs4994) polymorphism of the ADRB3 gene in the main group of pregnant women showed that the observed frequency of T/T genotypes was 11.4%, heterozygous T/C genotypes was 56.8 % and homozygous - C / C was 31.8%, respectively, while the expected frequency of genotypes of the T / T group and heterozygous genotypes -T / C was 46.8% and 43.2%, respectively, and homozygous C / C was in 9.9% of cases.

Table 4. Expected and observed distribution frequency of genotypes according to HWE of the T/Cr rs4994 polymorphism of the ADRB3 gene in the control group of pregnant women without MC

Genotypes	Frequency of genotypes		χ^2	P
	observed	expected		
T/T	28.6	50.2	0.250	**0.56**
T/C	42.8	41.3	0.5	
C/C	28.6	8.5	0.250	
total	100.00	100.00	0.0	

Whereas in the control group, the observed and expected frequency of functional T/T genotypes occurred in 28.6% and 50.0% of cases, respectively. The observed heterozygous genotype T/C was found in 42.8% of cases, and the expected one was 41.3%, while the frequency of the observed homozygous mutant C/C genotype was seen in 28.6% of cases, and the expected one was 8.5% of cases, respectively. (Table 4).

A comparative analysis of the expected and observed frequencies of the genotypes of this polymorphism revealed the absence of a statistically significant deviation of indicators (P> 0.05) in all the studied groups and subgroups (Table 4). This fact indicates that the observed proportion of genotypes in the studied samples corresponds to the Hardy-Weinberg equilibrium.

The analysis showed that both in the control and in the main groups with MC, the values of the expected and observed heterozygosity of the studied polymorphism had practically no differences. However, it should be said that in the main group of patients with MC in homozygous favorable T/T genotypes, a significant increase in the observed heterozygosity was found.

4. Conclusions

Thus, the data of our study showed the association of the “C” allele and the heterozygous genotype T/C of the rs4994 polymorphism of the ADRB3 gene with the development of miscarriage. At the same time, the risk of developing pathology in the carriage of the “C” allele and the T/C genotype increases by 1.5 (OR=1.51) and 1.8 (OR=1.75) times, respectively. The presence of the wild allele and genotype of the rs4994 polymorphism of the ADRB3 gene in a pregnant woman with MC plays a protective role in relation to the formation of MC.

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