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Prevalence of Some Gene Polymorphisms Related to Early Pregnancy Loss among Russian Women

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Abstract. Background. A variety of biological processes regulated by differential gene expression are required to maintain a normal gestation and accordingly, the mutations and polymorphisms in such genes may cause miscellaneous biological disorders that eventually result in early pregnancy loss. Many studies reported that aberrant fetal DNA methylation as well as embryonic chromosome abnormalities may lead to impairment of fetal early growth and development. Therefore, we have aimed to genotype several gene polymorphisms might be involved in the above-mentioned biological disorders to screen their prevalence in Russian population. Materials and methods. 81 Russian women without previous history of normal pregnancy or early abortion were recruited into this population study to determine the genotype and allele frequencies through genotyping using RFLP-PCR method for DNMT3B rs2424913, DNMT3B rs1569686, DNMT3A rs7590760, DNMT1 rs2228611, DNMT1 rs8101626, DNMT3L rs2276248, and DNMT3L rs2070565, allele-specific PCR for SYCP3 T657C, and real-time PCR for MTHFR rs1801133, MTHFR rs1801131, MTR rs1805087, and MTRR rs1801394. Results. Minor homozygous genotypes and minor alleles of the polymorphisms DNMT3B rs2424913 (TT: 11.1%, T: 37.05%), DNMT1 rs2228611 (GG: 18.5%, G: 40.75%), and DNMT1 rs8101626 (GG: 16.0%, G: 40.1%) were quite prevalent in Russian women and as frequent as those of the well-studied polymorphisms: MTRR rs1801394 (GG: 27.2%, G: 50.65%), MTHFR rs1801131 (CC: 17.3%, C: 40.15%), and MTHFR rs1801133 (TT: 11.1%, T: 29.0%).The heterozygous genotype of SYCP3 T657C (CT: 12.3%, T: 6.15%) was also quite frequent. Conclusion. Based on our study and literature data, we suggest that DNMT3B rs2424913, DNMT1 rs2228611, DNMT1 rs8101626, and SYCP3 T657C polymorphisms along with the common folate cycle gene polymorphisms can be potential genetic predictors for early pregnancy loss in Russian women.

Key words: gene polymorphisms, early pregnancy loss, DNA methylation, folate cycle, chromosome nondisjunction

Author Contributions. Ahmed A.A.M. — genotyping of study participants, writing text; Muradyan A.A. — collection of biological material; Azova M.M. — research concept and design.

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Встречаемость у женщин русской национальности некоторых генных полиморфизмов, ассоциированных с ранними репродуктивными потерями

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Аннотация. *Актуальность.* Разнообразные биологические процессы, регулируемые дифференциальной экспрессией генов, необходимы для нормального протекания беременности, и, соответственно, мутации и полиморфизмы в таких генах могут вызывать различные нарушения, приводящие, в конечном итоге, к преждевременной потере беременности. Во многих исследованиях сообщалось, что изменения в метилировании ДНК и хромосомные мутации могут привести к аномалиям развития плода. В этой связи представленное исследование было направлено на изучение распространенности в русской популяции ряда генных полиморфизмов, которые могут быть вовлечены в развитие указанных нарушений. *Материал и методы.* В исследовании принимали участие женщины русской национальности без беременности в анамнезе (n = 81). Генотипирование выполнялось методами ПЦР с последующей рестрикцией ДНК (DNMT3B rs2424913, DNMT3B rs1569686, DNMT3A rs7590760, DNMT1 rs2228611, DNMT1 rs8101626, DNMT3L rs2276248, DNMT3L rs2070565), аллель-специфичной ПЦР (SYCP3 T657C) и ПЦР в режиме реального времени (MTHFR rs1801133, MTHFR rs1801131, MTR rs1805087, MTRR rs1801394). *Результаты.* Минорные гомозиготные генотипы и минорные аллели по полиморфизмам DNMT3B rs2424913 (TT 11,1%, T 37,05%), DNMT1 rs2228611 (GG 18,5%, G 40,75%) и DNMT1 rs8101626 (GG 16,0%, G 40,1%) встречаются у женщин русской национальности достаточно широко, и их частота сравнима с таковой по хорошо изученным полиморфизмам MTRR rs1801394 (GG 27,2%, G 50,65%), MTHFR rs1801131 (CC 17,3%, C 40,15%) и MTHFR rs1801133 (TT11,1%, T 29,0%). Распространен также и гетерозиготный генотип по полиморфизму SYCP3 T657C (CT 12,3%, T 6,15%). *Заключение.* Результаты проведенных нами исследований и анализ литературных данных позволяют полагать, что генные полиморфизмы DNMT3B rs2424913, DNMT1 rs2228611, DNMT1 rs8101626 и SYCP3 T657C, наряду с хорошо изученными полиморфизмами генов фолатного цикла, могут быть использованы в качестве генетических предикторов ранних репродуктивных потерь у женщин русской национальности.

Ключевые слова: генные полиморфизмы, потеря беременности на ранних сроках, метилирование ДНК, фолатный цикл, нерасхождение хромосом

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Заявление о конфликте интересов. Авторы заявляют, что исследование проводилось при отсутствии какого-либо конфликта интересов.

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Introduction

Implantation of embryos occurs about 6 days after fertilization, and conception can be identified clinically after 5—6 weeks from the last menstrual cycle [1]. Around 80 percent of all pregnancy loss cases happen during the first-trimester [2]. When this problem occurs two or more consecutive times, it is known as recurrent pregnancy loss [3]. It has been observed that recurrent pregnancy loss (RPL) affects 2—5 percent of couples trying to have children [2]. Genetic factors causing RPL amount to 3—5 percent of cases, while around 7 percent of cases are due to cytogenetic abnormalities, 15 percent due to hormonal disorders, and 10—15 percent due to anatomical de-

fects [3]. It was reported that there are 30 or more genes exhibiting various expression levels between normal and RPL patients [4]. Therefore, interaction of several gene polymorphisms may lead to recurrent pregnancy losses.

DNA methylation is an epigenetic process of the covalent addition of a methyl group at the 5-carbon of the cytosine base yielding 5-methylcytosine in the dinucleotide sequence 5′CpG3′ which are primarily present directly upstream of gene promoters, thereby often influencing the function of the genes and moderating their expression [5]. DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs). DNMT3A and DNMT3B are taking charge of establishing *de novo* methylation patterns, whereas DNMT1 maintains these methylation patterns during mitotic division [5]. DNMT3L functions as an important cofactor to enhance the activity of DNMT3B and DNMT3A [6]. Mutations of the DNA methyltransferases may lead to aberrant DNA methylation resulting in early fetal death in mammals.

The DNA methylation is basically linked with methionine-homocysteine cycle in which folate mediates the methylation process of homocysteine to form methionine that is converted to s-adenosylmethionine,the major cellular methyl group donor required for genome-wide methylation process. Naturally, folate is biochemically inactive and needs first to be converted enzymatically to 5,10-methylenetetrahydrofolate. Methylenetetrahydrofolate reductase (MTHFR) then reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate,which functions as a methyl donor and plays an essential role in remethylation of homocysteine to methionine catalyzed by various enzymes particularly methionine synthase (MTR) and methionine synthase reductase (MTRR) [7—9]. Accordingly, MTHFR, MTR and MTRR are considered as significant regulatory enzymes involved in folate and methionine metabolism and critical mediators in DNA methylation. Moreover, folates have an important role in the re-methylation of plasma homocysteine to methionine to prevent the hyperhomocysteinemia and venous thrombosis that have been found associated with fetal loss as well [7, 10].

Fetal aneuploidy represents the most widespread cytogenetic abnormality in human early miscarriage. It arises due to the chromosome nondisjunction, which ultimately leads to early miscarriage or congenital birth syndromes. The maternal meiosis I errors are the leading cause of embryonic aneuploidy, which is found in 7 percent of RPL [11—13], whereas 50—70 percent found in sporadic spontaneous pregnancy losses [13]. Synaptonemal complex protein 3 (SYCP3) is an essential structural DNA-binding protein encoded by SYCP3 gene. SYCP3 is important for synapsis and recombination of homologous chromosomes in prophase of meiosis I. Normal SYCP3 self-assembles into cross-striated fibers that establish the lateral elements core of synaptic complex, which serves as scaffold to which other meiotic proteins connect [14]. The abnormality in this gene may often lead to an improper segregation of chromosomes, so SYCP3 T657C polymorphism might be related to recurrent pregnancy losses [15, 16].

According to the literature data, some studies revealed variations in the frequency of DNMT3B, DNMT3A, DNMT1, DNMT3L, and SYCP3 gene polymorphisms among different populations. These gene polymorphisms have been suggested being genetic risk factors for several diseases and reproductive disorders in women. MTHFR, MTR, MTRR are genes which polymorphisms have been extensively analyzed among the RPL women and were statistically associated with RPL. Virtually, these folate cycle gene polymorphisms are considered the most prominent genetic predictors used for early miscarriage.

In the present study we analyzed the following single nucleotide polymorphisms (SNPs) in Russian women from Central Russia: DNMT3B rs2424913, DNMT3B rs1569686, DNMT3A rs7590760, DNMT1 rs2228611, DNMT1 rs8101626, DNMT3L rs2276248, DNMT3L rs2070565, SYCP3 T657C, MTHFR rs1801133, MTHFR rs1801131, MTR rs1805087, and MTRR rs1801394. On one hand, as far as we

know, DNMT3B rs2424913, DNMT3A rs7590760, DNMT1 rs8101626, DNMT3L rs2276248, and DNMT3L rs2070565 polymorphisms have been analyzed only among women with various cancers and in male infertility [17—19]. DNMT3B rs1569686 and DNMT1 rs2228611 have been analyzed in cancerous disease but only one study has genotyped them in Slovenian women with RPL [20]. SYCP3 T657C has been analyzed among Iranian and Japanese women with idiopathic RPL [15, 16]. All those polymorphisms have never been yet investigated in Russian population. The novelty is that this study is the first one to assess the distribution of genotypes and alleles for these polymorphisms in Russian women. On the other hand, although the common folate cycle gene polymorphisms have been already investigated in Russian population, our study has aimed to reassess their genotype and allele distribution along with the new ones on purpose of comparing the distribution of their minor genotypes and alleles with those of new polymorphisms among Russian women.

Materials and Methods

The study was carried out on DNA samples from 81 women purely descending from Russian families and without any previous history of normal pregnancy or early pregnancy losses with a mean age of 22.3 ± 2.6 years. All individuals enrolled in this study gave informed consents for participation and processing of personal data. The study was approved by the Local Ethics Committee of the Institute of Medicine of RUDN University.

Genomic DNA was extracted from blood samples by standard procedures using a commercially available kit (Syntol, Russia). Storage of the extracted DNA was at −20 °C. Genotyping of DNMT3B rs2424913, DNMT3B rs1569686, DNMT3A rs7590760, DNMT1 rs2228611, DNMT1 rs8101626, DNMT3L rs2276248, DNMT3L rs2070565, and SYCP3 T657C SNPs was conducted using a Restriction Fragment Length Polymorphism-Polymerase Chain Reaction method (RFLP-PCR) and Allele-Specific PCR (Table 1). The amplified PCR products were digested with the suitable restriction enzymes (Sibenzyme, Russia). The resulting fragments were sorted and visualized by electrophoresis in 3.0% agarose gel containing ethidium bromide under ultraviolet light in reference to a molecular weight marker. Genotyping of MTHFR rs1801133, MTHFR rs1801131, MTR rs1805087, MTRR rs1801394 was performed using a commercially available kits for Real time-PCR (Syntol, Russia).

To analyze the genetic markers for the Hardy-Weinberg equilibrium the chi-square test was performed using the statistical software SPSS, version 22. P value ≤ 0.05 was considered statistically significant.

Genotype frequencies among the subjects for all gene polymorphisms except DNMT3L rs2070565 were in agreement with Hardy-Weinberg equilibrium (P value > 0.05). The genotype and allele frequencies of DNMT3B rs2424913, DNMT3B rs1569686, DNMT3A rs7590760, DNMT1 rs2228611, DNMT1 rs8101626, DNMT3L rs2276248, DNMT3L rs2070565, and SYCP3 T657C polymorphisms are stated in table 2. Our findings have revealed that the frequencies of minor homozygous genotypes and minor alleles of DNMT3B rs2424913 (TT: 11.1%, T: 37.05%), DNMT3B rs1569686 (TT: 16.0%, T: 39.5%), DNMT3a rs7590760 (GG: 24.6%, G: 50.55%), DNMT1 rs2228611 (GG: 18.5%, G: 40.75%), and DNMT1 rs8101626 (GG: 16.0%, G: 40.1%) polymorphisms were highly frequent in participants. Although the minor homozygous genotypes in SYCP3 T657C and DNMT3L rs2276248 weren't detected, their heterozygous genotypes (CT: 12.3%, CT: 6.2% respectively) were mildly frequent. Interestingly, our findings have revealed very high significant frequencies for DNMT3L rs2070565 heterozygous genotype and recessive allele (GA: 86.4%, A: 48.2%), while its minor homozygous genotype tended to be rare (AA: 4.9%). And as it has been mentioned before, its genotypes are not in Hardy-Weinberg equilibrium. This phenomenon needs to be studied more.

Таблица 1 / Table 1

Genotyping conditions / Условия генотипирования

Gene polymorphisms / Генные полиморфизмы	Primer sequences / Праймеры	PCR Protocol / Условия амплификации	Restriction enzymes / Рестриктазы	DNA fragments, bp / Длина фрагментов ДНК, пн
DNMT3B rs2424913	F: 5′-TGCTGTGACAGGCAGAGCAG-3′ R: 5′-GGTAGCCGGGAACTCCACGG-3′	–95 °C (5 min) –35x95 °C (30 s)/65 °C –(30 s)/72 °C (30 s) –72 °C (10 min)	ASPA2I	CC: 380 CT: 380, 207, 173 TT: 207, 173
DNMT3B rs1569686	F: 5′-GAGGTCTCATTATGCCTAGG-3′ R: 5′-GGGAGCTCACCTTCTAGAAA-3′	–94 °C (5 min) –35x94 °C (30 s)/49 °C –(30 s)/72 °C (30 s) –72 °C (5 min)	PVull	TT: 132, 93 TG: 225, 132, 93 GG: 225
DNMT3L rs2276248	F: 5′-TATGTTGTCCAGGCTCGTCTC-3′ R: 5′-ATCACAATCGCCAACCGTAG-3′	–94 °C (5 min) –35x94 °C (30 s)/56 °C –(30 s)/72 °C (30 s) –72 °C (5 min)	PVull	TT: 357 TC: 357, 218, 139 CC: 218, 139
DNMT3L rs2070565	F: 5′-GGGGTGCATCAGGGATCTGA-3′ R: 5′-CTAAGTGACTGGTCCAATAAGC-3′	–94 °C (5 min) –35x94 °C (30 s)/53 °C –(30 s)/72 °C (30 s) –72 °C (5 min)	ApeKI	AA: 218 AG: 218, 151, 67 GG: 151, 67
DNMT1 rs2228611	F: 5′-TATGTTGTCCAGGCTCGTCTC-3′ R: 5′-GTACTGTAAGCACGGTCACCTG-3′	–94 °C (5 min) –35x94 °C (30 s)/ 55 °C (30 s)/72 °C (30 s) –72 °C (5 min)	BStMAI	AA: 232, 28 AG: 232, 108, 124, 28 GG: 108, 124, 28
DNMT1 rs8101626	F: 5-CAAATGGGCCACCTAGACAC-3 R: 5′-GGCAGAGATTGAGCCAGAAG-3′	–94 °C (5 min) –35x94 °C (40 s)/ 67 °C (40 s)/72 °C (40 s) –72 °C (5 min)	BStMAI	AA: 640 AG: 640, 474, 166 GG: 474, 166
DNMT3A rs7590760	F: 5’-TGCTGTGCCTACTCCAAACA-3′ R: 5′-GCCATGAATGTCCAGAAGGT-3′	–94 °C (5 min) –35x94 °C (30 s)/62.6 °C (30 s)/72 °C (40 s) –72 °C (5 min)	RsaI	CC: 267, 76 CG: 267, 76, 343 GG: 343
SYCP3 (T657C)	F1 5′-ATGTTGCAAAAAAAAATTATGATGGAAGCT-3′ F2 5′-ATGTTGCAAAAAAAAATTATGATGGAAGCC-3′ R1,2 5′-TTGCTGCTGCTGTTTCATG-3′	–94 °C (5 min) –35x94°C (30 s)/ 60 °C (30 s)/72 °C (30 s) –72 °C (5 min)	—	TT: 286 CC: 286

Таблица 2 / Table 2

Genotype and allele frequencies (%) for DNMT1, DNMT3A, DNMT3B, DNMT3L, and SYCP3 gene polymorphisms / Частоты генотипов и аллелей (%) по полиморфизмам генов DNMT1, DNMT3A, DNMT3B и DNMT3L

Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %	Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %
DNMT3B rs2424913		**DNMT1 rs8101626**	
CC	37.0	AA	35.8
CT	51.9	AG	48.2
TT	11.1	GG	16.0
C	62.95	A	59.9
T	37.05	G	40.1
DNMT3B rs1569686		**DNMT3L rs2070565**	
GG	37.0	GG	8.6
GT	47.0	GA	86.4
TT	16.0	AA	4.9
G	60.5	G	51.8
T	39.5	A	48.2
DNMT3A rs7590760		**DNMT3L rs2276248**	
CC	23.5	TT	93.8
CG	51.9	CT	6.2
GG	24.6	CC	0.0
C	49.45	T	96.9
G	50.55	C	3.1

Окончание таблицы 2 / End of the table 2

Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %	Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %
DNMT1 rs2228611		**SYCP3 T657C**	
AA	37.0	TT	87.7
AG	44.5	CT	12.3
GG	18.5	CC	0.0
A	59.25	T	93.85
G	40.75	C	6.15

Таблица 3 / Table 3

Genotype and allele frequencies (%) for MTHFR, MTR, and MTRR gene polymorphisms / Частоты генотипов и аллелей (%) по полиморфизмам генов MTHFR, MTR и MTRR

Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %	Genotypes and alleles / Генотипы и аллели	Frequency, % / Частота, %
MTHFR rs1801131		**MTR rs1805087**	
AA	37.0	AA	61.7
AC	45.7	AG	33.3
CC	17.3	GG	5.0
A	59.85	A	78.35
C	40.15	G	21.65
MTHFR rs1801133		**MTRR rs1801394**	
CC	53.1	AA	25.9
CT	35.8	AG	46.9
TT	11.1	GG	27.2
C	71.0	A	49.35
T	29.0	G	50.65

Genotype and allele frequencies of MTHFR rs1801133, MTHFR rs1801131, MTR rs1805087, MTRR rs1801394 are displayed in table 3. These findings have detected high frequencies of minor genotypes and alleles for MTRR rs1801394 (GG: 27.2%, G: 50.65%), MTHFR rs1801131 (CC: 17.3%, C: 40.15%), and MTHFR rs1801133 (TT: 11.1%, T: 29.0%) whereas for MTR rs1805087 (GG: 5.0%, G: 21.65%) they were mildly frequent in participants.

Discussion

According to our findings, the recessive genotypes and recessive alleles for DNMT3B rs2424913, DNMT3B rs1569686, DNMT3a rs7590760, DNMT1 rs2228611, and DNMT1 rs8101626 were as roughly prevalent as those for MTRR rs1801394, MTHFR rs1801131, and MTHFR rs1801133, whereas they were more prevalent than those for MTR rs1805087 in Russian population. Globally several studies have detected associations between the MTHFR rs1801131, MTHFR rs1801133, MTR rs1805087, MTRR rs1801394 and recurrent pregnancy loss and have recommended to use them as routine genetic factors [21—27]. Referring to the studies mentioned above, respecting the significant association of the common polymorphisms with recurrent miscarriage, as well as to our findings, published in previous studies, in which we reported the statistical association of DNMT gene polymorphisms with early pregnancy loss [28, 29], and as all gene polymorphisms were similarly distributed among Russian women, we recommend considering the DNMT3B rs2424913, DNMT1 rs2228611 and DNMT1 rs8101626 SNPs as genetic markers for prediction of RPL in Russians.

On grounds of our findings about the SYCP3 T657C heterozygous genotype (CT) frequency, as well as the mentioned studies indicating the association of SYCP3 T657C with RPL in different populations [15, 16], we advise to analyze this polymorphism in larger samples to confirm its contribution to recurrent early miscarriage.

The results of our study were similar to the previous studies carried out on Iranian and European populations with respect to the genotype and allele frequencies of DNMT3B rs2424913, DNMT3B rs1569686, DNMT3a rs7590760, DNMT1 rs2228611, and DNMT1 rs8101626 [18, 20, 30, 31]. Likewise, the genotype and allele frequencies of DNMT3L rs2276248 in our study were consistent with those determined among healthy French women [32]. However, they were completely in contrast with the genotype and allele frequencies for DNMT3B rs2424913, DNMT3B rs1569686, DNMT3L rs2276248, and DNMT3L rs2070565 recorded among healthy Chinese population, which have been published in several case-control studies, and we expect that the cause of difference might be attributed to the ethnic factor [19, 33, 34]. The heterozygous genotype (CT) frequency for SYCP3 T657C was also comparable to that reported in an Iranian study [15]. Our findings also revealed that the frequencies of genotypes and alleles for MTHFR rs1801131, MTHFR rs1801133, MTR rs1805087, MTRR rs1801394 were alike to those detected previously in European and Russian populations [31, 35].

Conclusion

Based on the data stated in the present study and from the genetic standpoint that pregnancy loss is a polygenic problem, we recommend using DNMT3B rs2424913, DNMT1 rs2228611, DNMT1 rs8101626, and SYCP3 T657C polymorphisms along with the common polymorphisms as potential genetic factors to predict early pregnancy loss in Russian women.

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