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GENETICS

Association of *DNMT3B* and *DNMN3L* Gene Polymorphisms with Early Pregnancy Loss

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A total of 100 women with early pregnancy loss were recruited and further classified into two subgroups: sporadic pregnancy loss and recurrent pregnancy loss; each subgroup consisted of 50 women. The control group included 56 women with normal pregnancies. Genotyping was performed by PCR with restriction fragment length polymorphism analysis. A statistically significant increase in the frequencies of *TT* genotype and *T* allele for *DNMT3B rs2424913* polymorphism was found in the total patient group and in both patient subgroups in comparison with the control. Moreover, homozygous *TT* genotype was associated with increased risk of early pregnancy loss (both sporadic and recurrent). *DNMT3B rs2424913* gene polymorphism in women can be used a marker of predisposition to early pregnancy loss and recurrent pregnancy loss.

Key Words: *DNA methyltransferases; early pregnancy loss; single nucleotide polymorphisms*

Early pregnancy loss (EPL) is defined as the loss of a pregnancy within the first trimester of gestation; two or more spontaneous losses of clinically recognized embryo is considered as recurrent pregnancy loss (RPL) [4-6]. RPL is the most common early complication of pregnancy that affects 2-5% of couples trying to conceive [5]. Approximately 9-15% of all clinically diagnosed pregnancies eventuate in early spontaneous abortion [9]; 30% miscarriages occur in between implantation and gestation week 6, while after the first trimester, their frequency sharply decreases [4,9].

Failure of implantation and post-implantation development is the major cause of RPL and genetic factors are involved in 50-76% of cases. Several studies attempted to find genes regulating different aspects of the mother—fetus interaction. It was found that some variants in the promoter regions or splicing sites of genes can modulate the level of expression or activity of enzymes responsible for methylation patterns through *de novo* DNA methylation at the early stage of embryogenesis and during germ cell differentiation, which eventually leads to recurrent spontaneous pregnancy loss risk [15].

In mammals, DNA methylation plays a crucial role in the regulation of gene expression and embryonic development. Methyl groups are added to cytosines in the CpG sites by DNA-methyltransferases (DNMT). In humans, the DNMT family comprises several enzymes: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT3B and DNMT3A determine *de novo* DNA methylation patterns in embryonic implantation stage, while DNMT1 maintains and stabilizes these patterns during mitotic divisions. DNMT2 is a tRNA methyltransferase and has no effect on genomic DNA methylation [10]. *DNMT3L* exhibits no DNA methylation activity, but stimulates activity of *DNMT3B* and DNMT3A methyltransferases [11,14].

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We studied genetic association of single nucleotide polymorphisms in *DNMT3B* and *DNMT3L* genes (*DNMT3B* -149*C>T*, *DNMT3B* -579*G>T*, *DNMT3L rs2276248*, and *DNMT3L rs2070565*) with EPL including RPL. Although *DNMT3B* -149*C>T* polymorphism (*rs2424913*) has been analyzed in various cancers [7,13] and *DNMT3L rs2276248* and *DNMT3L rs2070565* polymorphisms have been studied in spermatogenesis disorders in men [7] and endometriosis in women [2], the association of these polymorphisms with early pregnancy loss have not been studied yet. *DNMT3B* -579*G>T* (*rs1569686*) was analyzed by one study in Slovenian women with recurrent pregnancy loss [1], but such a study has never been performed in Russia.

MATERIALS AND METHODS

We performed a case-control study to analyze possible association of gene polymorphisms *DNMT3B* -149*C>T rs2424913*, *DNMT3B* -579*G>T rs1569686*, *DNMT3L rs2276248*, and *DNMT3L rs2070565* with EPL in Russian women from Central Russia. Informed consent was obtained from all participants enrolled in the study.

The study included two groups. The patients group involved 100 women (mean age 31.5±4.9 years) with EPL before the 12th week of gestation. This group was divided into two subgroups: 50 women with sporadic pregnancy loss (SPL) occurring before having at least one viable fetus and 50 women with RPL. Exclusion criteria were anatomic abnormalities and chronic diseases that can induce EPL. The control group included 56 healthy women (mean age 29.2±3.5 years) who had at least one child from normal pregnancy and no history of pregnancy loss or any reproductive disorders.

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures using commercially available reagents (Syntol). Genotyping of *DNMT3B* -149*C>T* (*rs2424913*), *DNMT3B* -579*G>T* (*rs1569686*), *DNMT3L rs2276248* and *DNMT3L rs2070565* polymorphism was performed using PCR followed by restriction fragment length polymorphism (Table 1). DNA fragments were separated by agarose gel electrophoresis.

The results were processed statistically using SPSS Statistics 22. To compare genotype and allele frequencies between the studied groups, χ^2 and Fisher's exact test were used. Odds ratio (OR) and 95% confidence intervals (CI) were calculated.

RESULTS

Genotype and allele frequencies for *DNMT3B rs2424913*, *DNMT3B rs1569686*, *DNMT3L rs2276248*, and *DNMT3L rs2070565* in the studied groups are shown in Table 2.

We found that the distribution of *DNMT3B* -149*C>T* (*rs2424913*) genotypes and allele frequencies in the total EPL group and in SPL and RPL subgroups were significantly different from those in healthy women. In addition, the risk of EPL, SPL and RPL was higher in women with the minor homozygous *TT* genotype (OR 4.44; 4.78, and 4.1; 95%CI 1.48-13.32, 1.61-14.21, and 1.35-12.43, respectively). No significant differences in genotype and allele frequencies for other studied polymorphisms in all

TABLE 1. Genotyping Conditions

Gene polymorphism	Primer	Annealing temperature, °C	Restriction endonuclease	DNA fragment length, bp
DNMT3B rs2424913	F: 5'-TGCTGTGACAGGCAGAGCAG-3'	65	ASPA2I	*`CC*: 380
	R: 5'-GGTAGCCGGGAACTCCACGG-3'			*CT*: 380, 207, 173
				TT: 207, 173
DNMT3B rs1569686	F: 5'-GAGGTCTCATTATGCCTAGG-3'	49	PVuII	*TT*: 132, 93
	R: 5'-GGGAGCTCACCTTCTAGAAA-3'			*TG*: 225, 132, 93
				GG: 225
DNMT3L rs2276248	F: 5'-TATGTTGTCCAGGCTCGTCTC-3'	56	PVuII	*AA*: 357
	R: 5'-ATCACAATCGCCAACCGTAG-3'			*AG*: 357, 218, 139
				GG: 218, 139
DNMT3L rs2070565	F: 5'-GGGGTGCATCAGGGATCTGA-3'	53	ApeKI	*AA*: 218
	R: 5'-CTAAGTGACTGGTCCAATAAGC-3'			*AG*: 218, 151, 67
				GG: 151, 67

TABLE 2. Genotype and Allele Frequencies (%) for *DNMT3B rs2424913*, *DNMT3B rs1569686*, *DNMT3L rs2276248*, and *DNMT3L rs2070565* Gene Polymorphisms in the Studied Groups

Genotypes and alleles		Control (*N*=56)	EPL (*N*=100)	SPL (*N*=50)	RPL (*N*=50)
DNMT3B rs2424913	CC	55.4	30.0*	30.0*	30.0*
	CT	39.2	57.0*	56.0*	58.0*
	TT	5.4	13.0*	14.0*	12.0*
	C	75.0	58.5*	58.0*	59.0*
	T	25.0	41.5*	42.0*	41.0*
DNMT3B rs1569686	GG	39.2	35.0	30.0	40.0
	GT	42.9	49.0	56.0	42.0
	TT	17.9	16.0	14.0	18.0
	G	60.6	59.5	58.0	61.0
	T	39.4	40.5	42.0	39.0
DNMT3L rs2276248	TT	94.6	90.9	90.0	91.7
	CT	5.4	9.1	10.0	8.3
	CC	0	0	0	0
	T	97.3	95.4	95.0	95.8
	C	2.7	4.6	5.0	4.2
DNMT3L rs2070565	GG	12.5	6.0	10.0	2.0
	GA	71.4	81.0	76.0	86.0
	AA	16.1	13.0	14.0	12.0
	G	48.2	46.5	48.0	45.0
	A	51.8	53.5	52.0	55.0

Note. *$p \leq 0.05$ in comparison with control

groups of women with pregnancy loss and control group were revealed.

The role of DNMT and the dynamics and function of DNA methylation in embryonic stem cells and at the early stages of development have been analyzed in detail in mouse models and less so in humans. Some results pointed out that the *de novo* methyltransferase *DNMT3B* in human oocytes and embryos is crucial in the creation of DNA methylation patterns and participates in the establishment of a successful pregnancy in humans, particularly at the early stages of embryogenesis [12]. It has been previously demonstrated that DNMTB3 enzyme also plays a role in the maintenance and dynamic remodeling of DNA methylation patterns in differentiated cells [11]. Additionally, DNMT3L was found to enhance DNA methylation activity of DNMT3B by about 1.5-3 times [14]. These data prompted the researchers to analyze the correlation between maternal *DNMT* gene polymorphisms and early spontaneous abortion.

We found that the presence of homozygous *TT* genotype for the *DNMT3B* -149*C>T* polymorphism significantly increases the risk for early pregnancy loss. We propose that the contribution of male *DNMT3B* -149*C>T* genotype (*CT* or *TT*) is required to cause EPL, but further research is needed. Therefore, our findings are consistent with the results reported by several previous studies that *DNMT3B* is essential for normal embryogenesis. Moreover, up to 50% embryos lost during early spontaneous abortion have chromosome aberrations [1], while abnormal maternal global DNA methylation can affect chromatin structure and contribute to chromosome instability and non-disjunction during meiosis [3]. *DNMT3B* also plays an important role in regulating of the monocyte-macrophage differentiation at the fetal—maternal interface [9]. Thus, *T* allele of the *DNMT3B* -149*C>T* polymorphism increases not only the risk of cancer [13], but can be used as a marker of genetic predisposition to EPL and RPL.

The study preformed by Slovenian researchers showed that the frequency of the *DNMT3B rs1569686 GG* genotype and *G* allele in women with RPL was significantly higher that in controls [1], but we re-

vealed no association of this polymorphism with early pregnancy loss in Russian women.

DNMT3L stimulating DNT3B and DNMT3A activity is highly expressed in mature oocytes and during spermatogenesis and early preimplantation period [14]. Despite proven association of *DNMT3L rs2276248* and *DNMT3L rs2070565* polymorphisms with male infertility and ovarian endometriosis [2,8], we found no association between *DNMT3L* polymorphisms and EPL.

Our results suggest that the presence of *T* allele of *DNMT3B* -149*C>T* polymorphism in Russian women can be a predisposing factor for EPL. Therefore, this single nucleotide polymorphism can be used as a marker of individual predisposition to EPL including RPL.

REFERENCES

1. Barišić A, Pereza N, Hodžić A, Ostojić S, Peterlin B. A Single nucleotide polymorphism of DNA methyltransferase 3B gene is a risk factor for recurrent spontaneous abortion. Am. J. Reprod. Immunol. 2017;78(6). doi: 10.1111/aji.12765.
2. Borghese B, Santulli P, Héquet D, Pierre G, de Ziegler D, Vaiman D, Chapron C. Genetic polymorphisms of *DNMT3L* involved in hypermethylation of chromosomal ends are associated with greater risk of developing ovarian endometriosis. Am. J. Pathol. 2012;180(5):1781-1786.
3. Božović IB, Stanković A, Živković M, Vraneković J, Kapović M, Brajenović-Milić B. Altered LINE-1 methylation in mothers of children with Down syndrome. PLoS One. 2015;10(5). ID e0127423. doi: 10.1371/journal.pone.0127423.
4. Committee on Practice Bulletins — Gynecology. The American College of Obstetricians and Gynecologists Practice Bulletin no. 150. Early pregnancy loss. Obstet Gynecol. 2015;125(5): 1258-1267.
5. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspective. Int. J. Womens Health. 2017;9:331-345.
6. European Society of Human Reproduction and Embryology. Guideline on the management of recurrent pregnancy loss. URL: https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Recurrent-pregnancy-loss
7. Gao M, He D, Meng F, Li J, Shen Y. Associations of *DNMT3B* -149*C>T* and -2437T>A polymorphisms and lung cancer risk in Chinese population. World J. Surg. Oncol. 2016;14(1). ID 293.
8. Huang JX, Scott MB, Pu XY, Zhou-Cun A. Association between single-nucleotide polymorphisms of *DNMT3L* and infertility with azoospermia in Chinese men. Reprod. Biomed. Online. 2012;24(1):66-71.
9. Jeve YB, Davies W. Evidence-based management of recurrent miscarriages. J. Hum. Reprod. Sci. 2014;7(3):159-169.
10. Kim SY, Romero R, Tarca AL, Bhatti G, Kim CJ, Lee J, Elsey A, Than NG, Chaiworapongsa T, Hassan SS, Kang GH, Kim JS. Methylome of fetal and maternal monocytes and macrophages at the feto-maternal interface. Am. Reprod. Immunol. 2012;68(1):8-27.
11. Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat. Rev. Genet. 2018;19(2):81-92.
12. Petrussa L, Van de Velde H, De Rycke M. Dynamic regulation of DNA methyltransferases in human oocytes and preimplantation embryos after assisted reproductive technologies. Mol. Hum. Reprod. 2014;20(9):861-874.
13. Shen H, Wang L, Spitz MR, Hong WK, Mao L, Wei Q. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. Cancer Res. 2002;62(17):4992-4995.
14. Suetake I, Shinozaki F, Miyagawa J, Takeshima H, Tajima S. *DNMT3L* stimulates the DNA methylation activity of Dnmt3a and *DNMT3B* through a direct interaction. J. Biol. Chem. 2004;279(26):27,816-27,823.
15. Tajima S, Suetake I, Takeshita K, Nakagawa A, Kimura H. Domain structure of the Dnmt1, Dnmt3a, and *DNMT3B* DNA methyltransferases. Adv. Exp. Med. Biol. 2016;945:63-86.