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SHORT COMMUNICATIONS

DNMT1 and *DNMT3A* Gene Polymorphisms and Early Pregnancy Loss

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Abstract—DNA methyltransferase DNMT3A and DMNT1 are required for *de novo* and maintenance methyltransferase activities that catalyze the establishment of methylation patterns during embryogenesis and gametogenesis. Inactivation of their genes may cause embryonic lethality. We conducted a case-control study to explore the association between *DNMT3A rs7590760*, *DNMT1 rs2228611*, and *DNMT1 rs8101626* polymorphisms and early pregnancy loss susceptibility in Russian women. 100 women with early pregnancy loss (EPL) were involved and divided into two subgroups consisting of 50 women: sporadic pregnancy loss (SPL) and recurrent pregnancy loss (RPL). The control group included 56 women with full term pregnancies. Genotyping was performed using PCR-RFLP methods. *GG* genotype and allele *G* of *DNMT1 rs2228611* were significantly associated with EPL and RPL, and *GG* genotype of *DNMT1 rs8101626* with EPL, SPL and RPL. Our findings have shown that women carrying *GG* genotype of *DNMT1 rs2228611* had a higher risk of EPL and RPL (OR = 3.0, 95% CI: 1.44–6.23; OR = 3.94, 95% CI: 1.92–8.09 respectively) as well as that carrying GG genotype of DNMT1 *rs8101626* are at higher risk of EPL and RPL (OR = 2.64, 95% CI: 1.2–5.76). Conclusion: our results suggest that *DNMT1 rs2228611* and *DNMT1 rs8101626* gene polymorphisms are associated with early pregnancy loss and can be a genetic risk factor for recurrent pregnancy loss.

Keywords: early pregnancy loss, recurrent pregnancy loss, *DNMT1*, *DNMT3A*

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Early pregnancy loss (EPL) is a process in which a loss of clinical pregnancy occurs spontaneously before 12 weeks of gestation [1]. Recurrent pregnancy loss (RPL) is defined as the repeated occurrence of two or more expulsion of clinically recognized fetus [2, 3]. The prevalence of early pregnancy loss among all clinically diagnosed pregnancies is approximately 15%, while it accounts for 17–22% of all pregnancies [1, 4]. The rate of miscarriages that take place prior to 12 weeks of gestation is 80%, but sharply decreases after the first trimester [5]. A successful pregnancy occurs as a result of hormonal, immunologic, and cellular events required for fertilization, implantation, and embryonic development [6]. Failure of implantation and embryo development is a major cause of recurrent pregnancy loss caused by genetic factors that account for 50–76% of cases [1]. Although several embryonic and parental factors play a role in occurrence of repeated pregnancy loss, the underlying cause remains undetermined in up to 50% of cases and is still not sufficiently analyzed by the genetic studies [2, 7]. Several gene variants have been postulated that may impact either the expression or activity levels of DNA methyltransferases (DNMTs) responsible for *de novo* DNA methylation and maintenance of DNA methylation patterns at the early stage of embryogenesis and during germ cell differentiation that may lead to recurrent pregnancy loss [8]. Methylation of DNA at CpG islands plays an important role in the regulation of gene expression and genomic imprinting. This phenomenon occurs due to activity of DNA methyltransferases which use S-adenosylmethionine as a methyl group donor to form 5-methylcytosine. DNMT3A and DNMT3B are responsible for establishing *de novo* DNA methylation patterns. DNMT3L interacts with DNMT3A and DNMT3B and can increase the binding of DNMT3A/DNMT3B to DNA, thereby enhancing its activity [9]. DNMT1 is the chief enzyme that catalyzes the addition of the methyl group onto the unmethylated strand of the hemimethylated DNA molecule. DNMT1 is crucial for maintaining and stabilizing mammalian genome wide methylation patterns during the cell division [10].

The present study aimed to investigate the following single nucleotide polymorphisms (SNPs) in *DNMT3A* and *DNMT1* genes: *DNMT3A rs7590760*, *DNMT1 rs2228611*, and *DNMT1 rs8101626*. As far as we know, *DNMT3A rs7590760* and *DNMT1 rs8101626* polymorphisms have been only analyzed in women with ovarian cancer and human breast cancer [11, 12], the new here is that our study is the first one to analyze these two polymorphisms in women with EPL.

Table 1. Genotyping conditions

Gene polymorphisms	Primer sequences (Evrogen, Russia)	Annealing temperature	Restriction enzymes (Sibenzyme, Russia)	DNA fragments, bp
DNMT3A rs7590760	F: 5'-TGCTGTGCCTACTCCAAACA-3' R: 5'-GCCATGAATGTCCAGAAGGT-3'	62.6°C	RsaI	*CC*: 267, 76 *CG*: 267, 76, 343 *GG*: 343
DNMT1 rs2228611	F: 5'-TATGTTGTCCAGGCTCGTCTC-3' R: 5'-GTACTGTAAGCACGGTCACCTG-3'	55°C	BStMAI	*AA*: 232, 28 *AG*: 232, 108, 124, 28 *GG*: 108, 124, 28
DNMT1 rs8101626	F: 5'-CAAATGGGCCACCTAGACAC-3' R: 5'-GGCAGAGATTGAGCCAGAAG-3'	67°C	BStMAI	*AA*: 640 *AG*: 640, 474, 166 *GG*: 474, 166

DNMT1 rs2228611 has been analyzed in several studies in human ovarian and gastric cancers [11, 13, 14], as well as in human patients with oligospermia [15], but only one study in Slovenia analyzed it in women with RPL [10]. All those polymorphisms have never been investigated in any diseases in Russia to date. For all former reasons, these three SNPs were selected for the investigation to determine whether they can be associated with EPL and RPL in Russian women.

We performed a case-control study to investigate *DNMT* gene polymorphisms (*DNMT3A rs7590760, DNMT1 rs2228611*, and *DNMT1 rs8101626*) to know whether they are maternal risk factors for EPL and RPL in Russian women from Central Russia. The study was performed on DNA samples from 100 women with early pregnancy loss with a mean age of 31.5 ± 4.9 years. Patient group was classified into two subgroups: one subgroup with a sporadic pregnancy loss (SPL, $n = 50$) and the second subgroup with two or more consecutive pregnancy losses (RPL, $n = 50$), 21 of them didn't ever have viable fetus (primary RPL), 29 had at least one viable fetus before consecutive miscarriages (secondary RPL). Patients with anatomic abnormalities and chronic diseases which may induce EPL were excluded. 56 age-matched women (29.2 ± 3.5 years) recruited as a control group, they had healthy pregnancies and no past history of pregnancy loss or any other reproductive disorders.

Genomic DNA was extracted from peripheral blood leucocytes by standard procedures using a commercially available kit (Syntol, Russia). Genotyping of *DNMT3A rs7590760, DNMT1 rs2228611*, and *DNMT1 rs8101626* SNPs was conducted using a polymerase chain reaction-restriction fragment length polymorphism method (Table 1). The restriction fragments of PCR products were sorted by electrophoresis on 3.0% agarose gel.

The comparison of *DNMT3A rs7590760, DNMT1 rs2228611*, and *DNMT1 rs8101626* genotype and allele frequencies between studied groups was assessed using chi-square test. The chi-square test and Odds ratio with 95% confidence intervals (CIs) were performed using the statistical software SPSS, version 22.

The frequencies of analyzed genotypes and alleles among studied groups are shown in Table 2. Statistically significant difference was observed for the genotype distributions and *G* allele frequency for *DNMT1 rs2228611* polymorphism in EPL and RPL groups compared with the control one, but no significant difference was observed for women with SPL. In addition, the risk of EPL and RPL in women were highly elevated under the *GG* genotype (OR = 3.0, 95% CI: 1.44–6.23; OR = 3.94, 95% CI: 1.92–8.09 respectively). Moreover, it was observed that the frequency of *GG* genotype for *DNMT1 rs8101626* polymorphism in EPL, SPL and RPL was significantly higher when compared control subjects, but no differences were observed between the other genotype models (AA, *AG*). So women carrying *GG* genotype are at higher risk of EPL by 1.9 fold than non-carriers (OR = 2.64, 95% CI: 1.2–5.76). However, the *G* allele frequency was at a trend to have difference in women with EPL and RPL ($P = 0.071$; $P = 0.084$ respectively). Finally, there was no a statistical significant difference in the distribution of genotypes and alleles for *DNMT3A rs7590760* polymorphism in all groups of women with pregnancy loss compared with the control.

The program of embryonic development is organized by both genetic and epigenetic factors. DNA methylation is a major epigenetic factor that essentially contributes to the genome reprogramming during early embryogenesis and gametogenesis. After fertilization, maternal and paternal DNA methylation patterns formed in gametogenesis undergo progressive demethylation, the paternal genomic DNA is actively demethylated before replication starts, while the maternal genomic DNA is passively demethylated during every cell cleavage by the blastocyst stage, but are dynamically remethylated in post-implantation to support embryonic growth and differentiation [16]. Genetic studies have proven that both *de novo* and maintenance methyltransferase activities are required for the establishment of embryonic methylation patterns. DNMT3A and DNMT3B are crucial for *de*

Table 2. Genotype and allele frequencies (%) for *DNMT1 rs2228611*, *DNMT1 rs8101626*, and *DNMT3A rs7590760* gene polymorphisms in studied groups

Genotypes and alleles	Control ($n = 56$)	EPL ($n = 100$)	SPL ($n = 50$)	RPL ($n = 50$)
DNMT1 rs2228611				
AA	42.9	30.0*	38.0	22.0*
AG	44.6	40.0	38.0	42.0
GG	12.5	30.0*	24.0	36.0*
A	65.2	50.0*	57.0	43.0*
G	34.8	50.0*	43.0	57.0*
DNMT1 rs8101626				
AA	36.4	28.0	30.0	26.0
AG	52.7	48.0	46.0	50.0
GG	10.9	24.0*	24.0*	24.0*
A	62.75	52.0	53.0	51.0
G	37.25	48.0	47.0	49.0
DNMT3A rs7590760				
CC	32.2	26.0	32.0	20.0
CG	46.4	52.0	48.0	56.0
GG	21.4	22.0	20.0	24.0
C	55.4	52.0	56.0	48.0
G	44.6	48.0	44.0	52.0

* $p < 0.05$ in comparison with control.

novo methylation that establishes new embryonic methylation patterns and their inactivation causes embryonic lethality [17]. As described above, maintenance of DNA methylation patterns is fundamental for progressive development of embryo and DNMT1 is the key enzyme taking on this task. Two DNMT1 isoforms have been identified as a short form of DNMT1 (DNMT1o) produced in mature oocytes and pre implantation stage and a longer somatic DNMT1 isoform (DNMT1s) synthesized after fertilization to maintain global DNA methylation after embryo implantation [18]. It has been reported that after several rounds of cell division, 90% reduction of total methyl CpG occurs due to a complete inactivation of DNMT1 and homozygous DNMT1−/− embryos are arrested at late gastrulation stage and die during early embryonic development [17, 19]. These findings postulate that DNMT1 functions as a main maintenance methyltransferase in vivo.

Therefore, sequence variants of *DNMT1* may cause aberrant methylation patterns, including global DNA hypomethylation and gene-specific hypermethylation, giving rise to various diseases. Several studies have found an association between polymorphisms in *DNMT1* gene and several diseases, where *DNMT1 rs2228611* was associated with ovarian and breast cancer [11–14], gastric cancer [20], schizophrenia [21], and male infertility [15]. Moreover, recently, *DNMT1 rs2228611* was also evaluated to contribute for the risk of RPL in Slovenian [10]. *DNMT1 rs8101626* was linked to risk for human ovarian cancer [11]. Other *DNMT1* SNPs were also associated with mutagen sensitivity [22] and colorectal cancer [23].

Although epigenetic reprogramming has become recently the most important subject for research, which was extensively studied in mice, few data are obtainable about this process in human embryos. *DNMT1* is situated on human chromosome 19p13.2. *DNMT1 rs2228611* is a synonymous SNP located in exon 17 near the site of hnRNA splicing. The A>G substitution results in gaining of three exonic splicing enhancer motifs [21]. *DNMT1 rs8101626* polymorphism is located on intron 39 and suggested to affect the transcriptional regulation of *DNMT1* mRNA [24]. *DNMT3A* gene is situated on chromosome 2p23.3. *DNMT3A rs7590760* is an intronic single nucleotide polymorphism, and its influence on the gene expression is poorly understood. Our results indicated that *DNMT1 rs2228611* was strongly associated with RPL. Furthermore, we classified the RPL group into primary RPL and secondary RPL. It has been revealed that the frequency of the *DNMT1 rs2228611 GG* genotype was higher in primary (42.9%) than in secondary (31.0%) RPL and on contrary, the frequency of heterozygotes was higher in secondary RPL (48.3%) than in primary RPL (33.3%). We propose that miscarriage may also be dependent on the contribution of male *DNMT1* mutant allele to produce *DNMT1* embryos with both mutant allelic genes but this hypothesis needs to be studied. In addition, our findings reported a significant association between *DNMT1 rs8101626 GG* genotype and early pregnancy loss including RPL.

Generally, our results agree with a study found that DNMT1 and DNMT3A were both expressed in normal human villous and decidua and DNMT1 was significantly down regulated in EPL whereas DNMT3A did not change [25].

Thus, our results suggested that Russian women carrying *GG* genotype for *DNMT1 rs2228611* or *DNMT1 rs8101626* polymorphisms may have an increased genetic susceptibility to early pregnancy loss and recurrent pregnancy loss.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Local Ethics Committee of the Institute of Medicine of RUDN University and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all women involved in the study.

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