Ассоциация полиморфизма T657C гена SYCP3 с потерей беременности на ранних сроках у женщин русской национальности

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Актуальность. Анеуплоидия возникает вследствие нарушения расхождения хромосом, и большинство анеуплоидных эмбрионов погибают до 10 недели гестации, что приводит к потере на ранних сроках приблизительно 15% клинически диагностированных беременностей. Белок SYCP3 играет важную роль в конъюгации и рекомбинации гомологичных хромосом в первом мейотическом делении. Было показано, что отсутствие гена данного белка у мышей приводит к стерильности самцов и снижению фертильности самок вследствие нарушения расхождения хромосом. В этой связи *SYCP3* можно рассматривать в качестве кандидатного гена для оценки гипотезы о фетальной анеуплоидии, возникающей в результате мутаций генов, продукты которых участвуют в мейозе, и ведущей к ранним репродуктивным потерям.

Методы. В представленной работе был исследован полиморфизм *T657C* гена *SYCP3* у 100 женщин русской национальности с ранними репродуктивными потерями, подразделенных на две подгруппы: со спорадической потерей беременности (n=50) и привычным невынашиванием (n=50), а также у 56 фертильных женщин (контроль). Целью работы явилось изучение наличия ассоциации между данным полиморфизмом и ранними репродуктивными потерями в русской популяции. Геномная ДНК была выделена из периферической крови, генотипирование выполнялось с использованием аллель-специфической полимеразной цепной реакции. Для сравнения частот генов и генотипов в изучаемых группах применялись критерий Хи-квадрат и точный тест Фишера, для определения которых использовалось программное обеспечение SPSS Statistics, версия 22. Оно также было использовано для расчета отношения шансов (OR) и 95% доверительного интервала.

Результаты. Частота гетерозиготного генотипа *CT* была достоверно выше в группе женщин с ранними репродуктивными потерями и, в частности, в подгруппе со спорадической потерей беременности ($p<0,05$). Несмотря на тенденцию к увеличению частоты данного генотипа у женщин с привычным невынашиванием, статистически значимых отличий от контроля обнаружено не было.

Заключение. Полученные результаты позволяют предполагать наличие ассоциации между полиморфизмом *T657C* гена *SYCP3* и спорадической потерей беременности на ранних сроках.

Ключевые слова: SYCP3, генный полиморфизм, потеря беременности на ранних сроках, привычное невынашивание беременности.

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The Association of SYCP3 T657C Polymorphism with Early Pregnancy Loss in Russian Women

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Background. Aneuploidy is a consequence of the chromosome nondisjunction. Majority of aneuploid embryos die *in utero* between the 6th and 10th week, resulting in early pregnancy loss of approximately 15% of all clinically recognized pregnancies. SYCP3 plays an important role in pairing and recombination of homologous chromosomes in meiosis I, and it has been shown that the lack of this gene leads to sterility in male and subfertility in female mice due to chromosome nondisjunction. So *SYCP3* is a candidate gene to evaluate the hypothesis of fetal aneuploidy arisen from meiosis gene mutations as a cause for human early pregnancy loss.

Methods. In our study, the *SYCP3 T657C* polymorphism in 100 Russian women with early pregnancy loss (EPL) that were classified into 2 subgroups: with sporadic pregnancy loss (SPL, n=50) and recurrent pregnancy loss (RPL, n=50), as well as 56 normal fertile women (control), was examined to determine whether there is an association between the polymorphism and early pregnancy loss in Russian population. Genomic DNA was extracted from peripheral blood samples using the standard procedure of a com-

mercially available kit. Genotyping of *SYCP3* gene was determined by allele-specific polymerase chain reaction method. Data were analyzed using SPSS statistical software version 22. The chi-square test and Fisher's exact test were used to compare genotype and allele frequencies between analyzed groups. The odds ratio (OR) and 95% confidence intervals (CI) were used for risk estimation.
Results. Frequency of the heterozygous genotype (*CT*) was significantly higher in the group of women with early pregnancy loss as well as in women with sporadic pregnancy loss ($p<0.05$). In women with RPL, we found that the frequency of this genotype tended to increase but could not make a significant difference when compared with the control group.
Conclusion. Our findings postulate that the *T657C* polymorphism of the *SYCP3* gene is possibly associated with a sporadic early pregnancy loss.

Key words: SYCP3, SNP, early pregnancy loss, recurrent pregnancy loss.

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Introduction

A pregnancy loss is defined as the spontaneous termination of a gestation before the fetus becomes viable. Hence the term comprises all pregnancy losses from the time of conception until 6th month of gestation [1]. In the first trimester, early pregnancy loss, miscarriage and spontaneous abortion are interchangeable terms. Early pregnancy loss (EPL) is defined as the loss of a clinical pregnancy within the first 12 weeks of gestation and its frequency accounts for 9-17% for women aged 20–30 years, 20% – 40% at age 35 – 40 years and increasing to 80% at age 45 years [2]. Recurrent pregnancy loss (RPL) is a condition when a woman has two or more losses of clinically diagnosed first-trimester pregnancies [3]. Sporadic pregnancy loss (SPL) is a relatively common phenomenon; its prevalence is 15%–25% of all pregnancies [4]. RPL is attributed to various causes such as endocrine, genetic or structural abnormalities, infections, immunological disorders, and also unexplained causes [5]. Meiosis is essential process in the maturation of oocytes and there are a number of genes regulating this process, therefore a defect in one of the genes involved in meiosis might be the underlying cause of the reproductive problems of these patients, in particular aneuploidy in their offspring. More than 50% aneuploid embryos die *in utero* between the 6th and 10th week, resulting in early pregnancy loss of approximately 15% of all clinically recognized pregnancies. Predominantly, although it is considered as the leading cause of early pregnancy loss, the precise mechanism of aneuploidy remains poorly understood [4, 6].

Aneuploidy is defined as a numerical abnormality of chromosomes in the fetus. Fetal aneuploidy affects at least 5% of all pregnancies [5, 6]. Random fetal chromosomal abnormalities tend to affect 25%–50% of all women that experience sporadic miscarriages [4]. Many studies reported that aneuploidy is a consequence of the chromosome nondisjunction, it occurs when homologous chromosomes or sister chromatids fail to separate during meiosis I or II. The major mechanisms of nondisjunction that lead to aneuploidy are the meiotic events represented by synapsis between homologous chromosomes and cohesion between sister chromatids. Defects in genes controlling these events induce a greater susceptibility to nondisjunction and the generation of a fetal aneuploidy [5, 6]. Most human aneuploidies are prenatally initiated due to errors in the female meiosis I [4].

The synaptonemal complex (SC) is a tripartite structure that consists of two parallel axial or lateral elements (LE) and a central element (CE). The synaptonemal complex elements play a crucial role in promotion of pairing and recombination of homologous chromosomes during meiotic prophase I, this process is known as synapsis. In mammals, the SC includes synaptonemal complex proteins 1, 2, and 3 (SYCP1, SYCP2, and SYCP3) [7]. SYCP1 is an essential component of the transverse filament while SYCP2 and SYCP3 are both components of the axial/ lateral element of the SC. SYCP3 is a DNA binding protein located along the paired chromosomes and its activity regulates homologous chromosome synapsis [8].

The homozygous *SYCP3*- mutant male mice are sterile due to massive apoptotic spermatocyte death during meiotic prophase [7, 8]. It was noticed that the phenotype of male and female mice having a deficiency in SYCP3 were different. Whereas male mice were sterile as a result of maturation arrest of spermatogenesis, female mice were subfertile as a consequence of a severe oocyte pool reduction. Despite two thirds of fetuses were healthy, one third was

impacted by aneuploidy leading to cessation of embryo development *in utero* and ultimately to miscarriage [9].

Considering the recent study findings related to the role of *SYCP3* gene in reproductive status of mammals including human, we found that *SYCP3* is a strong candidate gene to evaluate the hypothesis of fetal aneuploidy arisen from meiosis gene mutations as a cause for human early pregnancy loss. *T657C* polymorphism (rs769825641, NM_001177949.2:c.657T>C) is one of the common mutations of the *SYCP3* gene affecting meiosis resulting in azoospermia in males and susceptibility to pregnancy loss in females due to aneuploidy [6, 8, 9, 10]. As far as we know, *T657C* polymorphism of the *SYCP3* gene was investigated by few studies in women with RPL [6, 8, 10], in infertile male [10] and in lung cancer [11]. Yet data presented about the implications of *SYCP3 T657C* polymorphism on early pregnancy loss are still conflicting and as well, it has not been analyzed in women with early pregnancy loss (EPL) in Russian population to date. Hence, we decided to examine it among women suffer from EPL and normal fertile women living in Central Russia.

Material and Methods

Participants and samples. We implemented a case-control study to evaluate the hypothesis pointing out that *T657C* polymorphism of *SYCP3* gene mutation can be a genetic risk factor for early pregnancy loss in humans. All the recruited subjects were Russians from Central Russia and gave informed consent for participation in the study. The study was approved by the Local Ethics Committee of the Institute of Medicine of RUDN University.

The study included the patient and control groups. The patient group with early pregnancy loss consisted of 100 patients with a mean of age 31.5±4.9 years was classified into two subgroups: with sporadic pregnancy loss (n=50) that experienced a single first-trimester pregnancy loss and had at least one normal birth later, and recurrent pregnancy loss (n=50) that had at least two or more first-trimester pregnancy losses. After being examined by specialists, the etiology of pregnancy loss could not be explained by classical criteria for EPL evaluation as well as patients with chronic diseases were excluded from the study. 56 healthy age matched women (29.2±3.5 years) that had at least one child from a normal pregnancy and no previous history of pregnancy loss or any other reproductive disorders were selected as a control group.

DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood samples using the standard procedure of a commercially available kit (Syntol, Russia). Presence of *T657C* mutation in coding exon 8 of *SYCP3* gene was determined by Allele-Specific polymerase chain reaction method using the following primers: F1 5'ATGTTGCAAAAAAAATTATGATGGAAGCT3', F2 5'ATGTTGCAAAAAAAATTATGATGGAAGCC3', and R1,2 5'TTGCTGCTGCTGTTTCATG3' [8].The amplification was performed in a total volume of 25 μL PCR Master Mix using Bio-Rad CFX96 system. PCR cycle conditions consisted of an initial melting step of 94°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing temperature at 60°C for 30 s, and 72°C for 30 s for extension, and a final extra extension step of 5 min at 72°C. The amplified DNA fragment (286 bp) was visualized on 2.0 % agarose gel.

Statistical analysis. Data were analyzed using SPSS statistical software version 22. The chi-square test or Fisher's exact test was used to compare genotype and allele frequencies between analyzed groups. The odds ratio (OR) and 95% confidence intervals (CI) were used for risk estimation. P value less than 0.05 was considered as statistically significant.

Results

The distribution of different genotypes and allele frequencies of *SYCP3 T657C* polymorphism in women with SPL were significantly different compared with the control group as well as the genotype frequencies in EPL but not the allele frequencies (Table 1).

We revealed that the heterozygous genotype leads to almost 1.7-fold increased risk of EPL (OR 3.45, 95% CI: 1.4 – 8.52) and SPL (OR 4.1, 95% CI: 1.71 – 10.15) among Russian women.

Discussion

Genetic studies analyzed small number of genes that might be essential for initiation, progression or completion of meiosis and their mutations ultimately may result in an abnormal chromosomal constitution of germ cells. After zygote formation by these germ cells, non-implantation or miscarriages might be the consequence. SYCP3 encodes a DNA binding protein that aids in the SC formation during meiosis I. It

Table 1

Genotype and allele frequencies (%) for *SYCP3 T657C* gene polymorphism in studied groups

Genotypes and alleles	Control (n=56)	EPL (n=100)	SPL (n=50)	RPL (n=50)
TT	91.1	78.0*	74.0*	82.0
CT	7.1	21.0*	24.0*	18.0
CC	1.8	1.0	2.0	0.0
T	94.65	88.5	86.0*	91.0
C	5.35	11.5	14.0*	9.0

Note. *p<0.05 in comparison with control.

was reported that the defect in *SYCP3* gene yield to failure of meiotic chromosome segregation (aneuploidy) due to nondisjunction [9]. The abnormal oocyte karyotype is inherited by embryos that die *in uterus* at an early stage of development. Fetal aneuploidy affects at least 5% of all pregnancies, and 25% - 50% of all sporadic miscarriages [4, 5, 6].

Several studies have been conducted to determine the role of *SYCP3* gene in meiosis. These studies have shown that offspring derived from chromosomally abnormal oocytes of null mice (*SYCP3−/−*) died *in uterus* as a consequence of fetal aneuploidy [9]. In addition, it has been proven that recombination errors occur due to inefficiently repaired DNA double stranded breaks in *SYCP3−/−* oocytes, thereby generating offspring which died *in uterus* [12]. Yuan et al. showed that *SYCP3−/−* male mice are sterile due to massive apoptotic spermatocyte death during meiotic prophase [7].

In the present study, we evaluated the presence of the mutation of *SYCP3* gene in Russian women, which might be associated with increased risk for early pregnancy loss. Our findings suggest that the minor *C* allele of the *SYCP3 T657C* polymorphism may be a genetic prognostic factor for random unexplained early pregnancy loss but not for the recurrent pregnancy loss. Our findings coincide with the study implemented in 2009 by Hsbaira Bolor et al. in Japan. H. Bolor identified two common and functional *SYCP3* gene mutations (*IVS7-16-19 del ACTT* and *T657C*), where it was found that two out of 26 women with unexplained recurrent pregnancy loss carried heterozygous nucleotide alterations in *T657C* polymorphism that caused early recurrent pregnancy loss, neither of which was reported in a group of healthy control [6].

In women with RPL, we found that the frequency of the heterozygous *CT* genotype tended to increase but could not make a significant difference when compared with the control group. The literature data about such association are controversial. E. Mizutani et al. in 2011 found that *CT* genotype was not associated with recurrent pregnancy loss [13] whereas Sazegari A. et al. revealed that the frequency of the heterozygous genotype and allele *C* in Iranian women with recurrent pregnancy losses were significantly higher than in the control group [8]. It is also possible that not all aneuploid conceptuses undergo early miscarriage; some of them may be lost in the preimplantation stage or after the first trimester.

Our results are supporting the prevailing hypothesis that recurrent miscarriage may be a polygenetic disorder influenced by both genetic and environmental factors. Moreover recurrent pregnancy loss also may arise from multiple mutations and polymorphisms in different genes contributing to embryo implantation and development. To a certain degree, our findings do not conflict with those studies demonstrating relationship of the *SYCP3 T657C* polymorphism with recurrent pregnancy losses because our results indicated a tendency to increased frequency of *CT* genotype in women with RPL. Therefore, we think that due to the complexity of meiosis and the variety of genes involved in this process, it is necessary to conduct more studies on such genes as well as the *SYCP3 T657C* polymorphism within bigger samples in order to determine whether it is a risk for RPL.

Conclusion

In our study, we evaluated the possible contribution of *SYCP3 T657C* polymorphism to the risk for EPL. Higher frequency of *C* allele and *CT* genotype in the patient group shows the association between *SYCP3 T657C* SNP and early pregnancy loss. Our results indicate that SC proteins may be associated with the random fetal chromosomal abnormalities that cause sporadic miscarriages.

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