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GENETICS

The T657C polymorphism on the SYCP3 gene is associated with recurrent pregnancy loss

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Abstract SYCP3 (Sinaptonemal complex protein 3) plays a critical role in pairing and recombination of homologous chromosomes in meiosis 1. It has been shown that lack of this gene leads to infertility in male and weakened fertility in female mice. In a case–control study, we investigated the SYCP3T657C polymorphism in the genome of 100 Iranian women with recurrent pregnancy losses of unknown causes as well as 100 control samples of normal fertile women having at least one healthy child. The general aim of our study was to determine whether there is a relationship between genetic changes in the SYCP3 gene and recurrent pregnancy loss in human or not. Frequency of the heterozygous genotype and mutated allele C were significantly higher in women with recurrent pregnancy losses (P-value<0.005). Our findings suggest that the T657C polymorphism of the SYCP3 gene is possibly associated with recurrent pregnancy loss of unknown cause in human.

Keywords Recurrent pregnancy loss · Sinaptonemal complex protein 3 · Polymorphism · Allele-Specific PCR · Meiosis

Capsule Frequency of SYCP3 657C allele were significantly higher in women with recurrent pregnancy losses suggested the T657C polymorphism of the SYCP3 gene is possibly associated with recurrent pregnancy loss of unknown cause in human.

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Introduction

Recurrent pregnancy loss as one of the most common fertility problems in women, is defined as two or more consecutive pregnancy losses, implicating 0.5–3 % of couples [1, 2]. Recurrent pregnancy loss may result from various causes such as genetic or structural abnormalities, infections, immunological causes, and also unknown causes [3]. Among genetic abnormalities, fetal chromosome aneuploidies including trisomy or monosomy of fetal chromosomes or fetal autosomal disorders are the most common causes of early recurrent pregnancy losses. These abnormalities occur as a common problem of human fertility in at least 5 % of all pregnancies [4]. Some aneuploid fetuses survive for a while, but suffer from several congenital anomalies. However, most of these fetuses die in the uterus that results in early pregnancy losses. In addition, about 15 % of all clinically-diagnosed abnormal pregnancies are lost between 6 and 10 gestional weeks, more than 50 % of which are due to numerical chromosomal abnormalities [3, 5]. Although these numerical chromosomal abnormalities are the main cause of early pregnancy losses, their underlying mechanisms are not well recognized. In fact, recurrent pregnancy loss is a serious fertility problem involving almost 5 % of couples trying to conceive [5]. Although recent theories suggest that recurrent pregnancy loss is probably a multifactorial abnormality influenced by both genetic and environmental factors, it is also likely to be associated with single-gene disorders. In this case, genes involved in chromosome pairing during meiosis 1 and crossing over are considered as biological candidates causing synaptic errors (error in homologous chromosomes pairing) and their consequent aneuploid gametes and zygotes [6]. Synaptonemal complex (SC) contains a tripartite protein structure that directs homologous chromosomes binding during prophase I of meiosis [7, 8]. SYCP3 is a DNA binding protein involved in the SC formation during meiosis 1, and its activity leads to

homologous chromosomes pairing and meiotic homologous recombination. SYCP3 is a fundamental component of SC structure that holds homologous chromosomes together [9]. Considering the recent findings about the role of SYCP3 gene in reproductive status of mammals including human, in this study we evaluated the hypothesis that SYCP3 gene mutations in human can be genetic risk factors causing reproductive disorders in women. Through a case–control study, we examined the frequency of one of the common and functional mutations of this gene i.e. T657C among women with recurrent pregnancy loss and compared this frequency with normal fertile women.

Materials and methods

In this study, 100 women of 25–35 years old with recurrent pregnancy loss with a history of at least two pregnancy losses of unknown cause were successively selected; after being examined by specialists, cases with issues such as induced pregnancy loss, infection of systemic diseases, uterine structural abnormalities, and personal or family history of thrombosis as well as patients with chromosomal abnormalities in their medical records were excluded from the study. Also, as control group, 100 normal women with at least one child from a normal pregnancy and no history of pregnancy loss were selected for sampling. Cases and controls were matched according to their age.

The study was approved by the ethics committee of recurrent abortion clinic of Yazd Reproductive Sciences Institute, which is a referral center for reproductive disorder, and our patients came from all over the Iran.

After full explanation about the study and taking written consents from both groups, 5 ml of venous blood was collected from each patient using EDTA (ethyinene diamine tetra acitic acid) anticoagulant to prevent blood clotting. Genomic DNA was isolated from peripheral blood samples using the standard salting-out procedure and kept in good conditions.

Presence of T657C mutation in SYCP3 gene was evaluated by Allele-Specific PCR method using the primers showed in Table 1.

The amplification was accomplished with a 25 μl reaction mixture containing 100 ng DNA, 40 pmol of primers SYCP3 F1, F2 and SYCP3 R1,R2 and 12.5 μl 2xTaq PCR Master Mix. The amplification was carried out with an initial melting step of 94 °C for 5 min; followed by 35 cycles of 94 °C for 30 s, annealing temperature starting at 60 °C for 30 s, and 72 °C for 30 min for extension, and a final elongation step of 5 min at 72 °C. The amplified DNA was visualized on 1.0 % agarose gel containing ethidium bromide.

The chi-square test was used to compare allele and genotype frequencies between case and normal groups. Statistical tests of significance and $\chi 2$ analysis were performed using SPSS statistic software version 18.0. The differences in allelic and genotypic frequencies of T657C locus in the SYCP3 gene between case and control groups were evaluated by $\chi 2$ test with odds ratio (OR).

Deviation from Hardy-Weinberg equilibrium was tested using HWE software.

Investigation of T657C polymorphism in SYCP3 gene using ARMS PCR method and the abovementioned primers is as follows (Fig. 1):

CC Genotype: 286-base-pair band in tube C
TT Genotype: 286-base-pair band in tube T
CT Genotype: 286-base-pair band in both tubes

Results

Frequencies of TT and CC+CT genotypes (CC=0, CT=18) for T657C polymorphism were 82 and 18 % in the case group as well as 92 and 8 % (CC=0, CT=8) in the control group, respectively, which were significantly different between the two groups (P=0.036). Frequencies of T and C alleles for this polymorphism were 0.91 and 0.09 for the case group as well as 0.96 and 0.04 for the control group, respectively, which were again significantly different between the two groups (Table 2).

Discussion

SYCP3 is a complex protein that allows homologous chromosomes pairing and recombination in synaptonemal structure of meiosis 1. SYCP3 is an essential component of axial and lateral

Table 1 primers used for amplification of exon 8 of SYCP3 gene and length of the products

Subjected exon	Orientation	Primer sequence	PCR product (base pair)
Exon 8	F1	5′-ATGTTGCAAAAAAAAATTATGATGGAAGCT-3′	286
	F2	5′-ATGTTGCAAAAAAAAATTATGATGGAAGCC-3′	286
	R1,2	5′-TTGCTGCTGCTGTTTCATG-3′	286
Control	F	5′-AGTATTTTGCCCACTAAATTGGA-3′	901
	R	5′-TTGCTGCTGCTGTTTCATG-3′	901

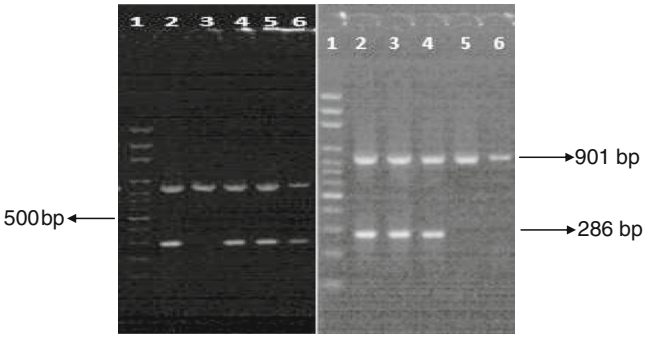

Fig. 1 The (AS-PCR) analysis of SYCP3T657C polymorphism. Both C and T alleles generated 286 bp bands that are the product of the Allele-Specific PCR reactions by the AS primers. The 901 bp bands are related to participating of control primers in the PCR reactions which must always be present. The left picture is related to PCR reaction with C primer and the right picture is related to PCR reaction with T primer. Similar numbers in each picture are products of PCR reaction with the same samples. 2 and 4 = CT genotypes, 5 and 6 = CC genotype, 3 = TT genotype and 1 = 100 bp DNA ladder

elements of SC [10]. Several studies have been conducted to examine the role of SYCP3 gene in meiosis and as a result, the reproductive status of mammals including human; one of the basic strategies in these studies is to generate SYCP3 gene-deprived mice. These studies show that in female mice null (SYCP3−/−) chromosomes are unable to form the synapsis of meiosis [11]. Fertility is weakened in these mice because of their oocyte reserve reduction [12]. In addition, some of the previous researches indicate that common and functional mutations of IVS7-16-19 del ACTT and T657C in the human SYCP3 gene in women cause early recurrent pregnancy loss [13].

In 2005, in a study by a group of Swedish scientists it was shown that degradation of SYCP1 gene in male mice leads a large number of spermatocytes to stop in pachytene stage of meiosis 1 and become infertile. Therefore, it was suggested that SYCP1 gene had a coordinating role in synapse formation as well as homologous chromosomes pairing [14]. In 2002, Li Yuan et al. conducted a comprehensive study on mouse SYCP3 gene: high expression of SYCP3 protein in SC demonstrates its strong role in this complex [15]. Their analysis on SYCP3-containing spermatocytes at different stages of meiosis using silver staining confirmed that SYCP3 is a fundamental component of the axial and lateral elements that holds homologous chromosomes together. They concluded that SYCP3 is essential for both formation and maintenance of these elements in SC structure. Yuan showed that SYCP3−/− male mice are infertile because of loss of germ cells in meiotic zygotene stage and activation of apoptotic cell death mechanism. Different studies have revealed that the inability to progress in meiosis is due to lack of DMC1, ATM, and SYCP3 proteins in spermatocytes [16, 17].

The first study on human SYCP3 was conducted in 2009 by Hsbaira Bolor et al. in Japan. They studied 26 women with recurrent pregnancy loss of unknown cause and detected two different homozygous mutations in SYCP3 gene of two different women. Bolor sequenced all the samples and identified the two mutations. One of them was IVS7-16-19 del ACTT in SYCP3 gene in a 39-year-old woman with a history of three consecutive pregnancy losses between 6 and 10 week of the pregnancies and the other mutation was T657C in a 29-year-old with a similar history of consecutive pregnancy losses [13]. They showed that although SYCP3 T657C mutation does not affect the encoded amino acid at this position (Thr), it is located at the extreme end of the exon and thus might affect normal splicing, inducing exon skipping or partial intron inclusion via a cryptic splice site in the intron. Therefore this mutation probably has a strong effect on SYCP3 protein function and is one good candidate for further evaluation.

Eita Mizutani et al. in 2011 conducted a study on SYCP3 gene in Japan. They investigated T657C mutation of SYCP3 gene in genomes of 101 patients with recurrent pregnancy loss of unknown cause as well as 82 normal fertile women without history of pregnancy loss as control group. In this study, they detected the heterozygous mutation of T657C in exon 8 of SYCP3 gene in a patient and a fertile control woman. However, the effects of SYCP3 mutations on the SC function or chromosomes non-disjunction in mammals have not been clarified [18].

Table 2 Genotypes and allele frequencies of SYCP3 T657C polymorphism in infertile females with recurrent pregnancy loss and fertile controls

P value	OR(95 %Cl)	Control=100	Case=100	T657C
0.036	0.3961(0.163–0.959)	92	82	TT
–	–	8(CC=0,CT=8)	18(CC=0,CT=18)	CC+TC
0.043	0.4213(0.178–0.992)	192	182	T
	Base	8	18	C

Mizutani reported that a patient with recurrent pregnancy loss and T657C mutation has normal karyotype and T657C mutation might be a functionless polymorphism investigated by Bolor in human. He stated that to determine whether mutations of SYCP3 can cause recurrent pregnancy loss, further studies are required with a greater sample size and a wide range of patients.

Mizutani mentioned that to increase our knowledge about the molecular mechanisms involved in recurrent pregnancies loss, routine screening of SYCP3 mutation in women with recurrent pregnancy loss requires further studies on animal models and human.

Due to complexity of meiosis as well as the variety genes involved in this process, it seems unlikely that mutation in a single gene is the cause of recurrent pregnancy loss in a high number of patients. Multiple mutations and polymorphism in different genes may result in chromosomes non-disjunction and consequently cause recurrent pregnancy loss [19].

According to the abovementioned subjects from different studies, some of them have strongly indicated the relationship between mutations of SYCP3 with recurrent pregnancy loss, while some others have rejected that.

In this study, based on evaluation of 100 patients with pregnancy loss of unknown cause and 100 women with normal fertility, we investigated the hypothesis that presence of functional mutations of SYCP3 gene is associated with increased risk of recurrent pregnancy loss in women.

In fact in this study, the relationship between SYCP3 T657C polymorphism and infertile women with recurrent pregnancy loss in Iran has been evaluated. Because the results obtained in polymorphism studies could vary with different racial and geographic situations of the study population, it was evaluated whether these mutations could be a genetic risk factor for the development of recurrent pregnancy loss in Iranian women or not. Our results showed that in Iranian women, this polymorphism is associated with an increased risk of recurrent pregnancy loss. The data and its association with RPL obtained in this study suggest that SYCP3 T657C polymorphism may participate in the etiology of RPL by affecting homologous chromosome pairing during meiosis I. Our findings confirm the results of previous studies indicating the relationship between this polymorphism and fertility weakness as well as recurrent pregnancy loss.

Conclusion

In our study the statistical analysis performed on the genotype frequency of SYCP3T657C polymorphism indicated that frequency of CC+TC genotype was 8 % in normal and 18 % in recurrent pregnancy loss groups (P Value<0.05). Therefore, higher frequency of C allele and TC genotype in the patients group shows the association between single nucleotide polymorphism and recurrent pregnancy loss in the study population. However, this significant difference between allelic and genotypic frequencies is not clinically useful in diagnosing the etiology of that individuals recurrent losses and further studies on other polymorphisms in SYCP3 gene and evaluation of Interaction between them are necessary to achieve the etiological role of SYCP3 in RPL.

Regarding the verified role of SYCP3 protein in SC structure, homologous chromosomes pairing, and meiotic recombination, our results seem biologically logical and indicate that SC proteins have important roles in meiosis and chromosomes pairing in mammals, and there is a relationship between T657C polymorphism of SYCP3 gene and women with recurrent pregnancy loss; therefore, the mentioned polymorphism can be considered as a genetic factor for pregnancy loss in human.

Further functional studies are required to reveal new evidence of human SYCP3 protein role in pathogenesis of recurrent pregnancy loss in women; those would be helpful in detection of SC mechanisms as well as homologous chromosomes pairing in mammals.

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