



Glycodelin (*PAEP*) gene promoter polymorphism rs760140467 C>T is associated with recurrent pregnancy loss

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ABSTRACT

In the absence of confirmed causes for around 50% of recurrent pregnancy loss (RPL) cases this study was conducted in order to evaluate the relationship between *PAEP* gene single nucleotide polymorphism (SNP) rs760140467 C>T and recurrent pregnancy loss (RPL) in Palestinian women. A retrospective case-control study was carried out during the period (August 2015 to April 2016). A total of 200 females, 100 RPL patients and 100 control women without previous history of RPL were included in the study. *PAEP* (rs760140467 C>T) polymorphism was tested by PCR-RFLP. Statistically significant difference existed between RPL cases and controls in terms of the genotypic and allelic distribution of the tested polymorphism. *PAEP* "T/C" genotype and "T" allele were significantly higher in the RPL group. The study showed, for the first time, that the "C/T" genotype and the "T" allele of the tested polymorphism are strongly associated with RPL in the investigated population. This finding may lead to improved therapeutic approaches for those RPL cases.

Keywords: *PAEP* gene, rs760140467 C>T , polymorphism, recurrent pregnancy loss.

INTRODUCTION

Glycodelin, the product of *PAEP* (Progestagen-Associated Endometrial Protein) gene on chromosome 9q34.3, is secreted by the uterine endometrium under progesterone induction, and is the most abundant progesterone-regulated secretory glycoprotein of the uterus at the time of implantation and early pregnancy. Glycodelin is inhibitory to the activity of T, B, NK cells and monocytes [1-3] and significantly contributes to immunomodulation towards fetal tolerance [4]. Importantly, subnormal levels of glycodelin correlates with recurrent miscarriage [5].

Several investigators have assessed the possible association between RPL and immune tolerance-related genes' SNPs, including cytokines e.g., *IL-10* [6], receptors e.g., *CTLA-4* [7] and transcription factors e.g., *FOXP3* [8]. Results of those studies suggest that such SNPs may confer susceptibility to RPL by modulating the immune system at the materno-fetal interface.

Due to its important immuno-modulatory role during early phases of pregnancy, this study was undertaken in order to test, for the first time, whether *PAEP* promoter "rs760140467 C/T" SNP is associated with RPL. The reason behind selecting this particular SNP is that it is located in a plausible progesterone receptor binding motif.

MATERIAL AND METHODS

Selection of *PAEP* promoter SNP rs760140467 C>T

The *PAEP* gene promoter sequence was first retrieved from the promoter database (<https://cb.utdallas.edu/cgi-bin/TRED/tred.cgi?process=searchTF> GeneForm) and then searched for progesterone receptor (PR) binding motifs [9]. The two PR binding sites found were then searched for single nucleotide variation by consulting Ensembl

database (http://asia.ensembl.org/Homo_sapiens/Info/Index), and the SNP rs760140467 (-468 C>T) was thus selected for genotyping .

Study population

The study was conducted on 100 Palestinian women, 18–35 years old, who had at least two RPLs ≤ 20 weeks of gestation. Age and ethnicity matched 100 women with at least two live births and without a previous history of abortion or pregnancy-associated complications served as the control group.

Ethical Considerations

Informed consent was obtained from all participants and the study procedure was approved by the local ethics committee.

DNA extraction

The DNA was isolated from whole blood samples using Wizard DNA extraction kit (Promega, USA) as described by the manufacturer. The isolated DNA was stored at -20°C until analysis of the polymorphism.

Analysis of PAEP gene SNP by rs76014067 T>C PCR-RFLP

The PAEP SNP rs76014067 T>C was genotyped using PCR-RFLP protocol. The primers were designed using online Primer3 software (<http://primer3.ut.ee/>) based on the genomic sequence deposited in the NCBI gene bank and the SNP sequence was retrieved from NCBI-SNP database.

Then restriction enzyme "BciVI" required for the PCR-RFLP identification of the SNP alleles was selected from new England Biolabs web site (<http://nc2.neb.com/NEBcutter2/>). The PCR primers, restriction enzyme digestion results and interpretation were done as depicted in Table-1.

The PCR reaction was carried out in a total volume of 20 μL , with 10 μL Taq PCR Master mix (Promega, USA), 2 μL (10pmol) of each primer, 4 μL nuclease-free water and 2 μL (50ng) genomic DNA. The PCR protocol was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation for 1 min at 94°C , annealing for 45 sec at 57°C and extension for 50 sec at 72°C . Final extension was carried out for 5 min at 72°C . The PCR 182 base pairs (bp) product size was confirmed with electrophoresis in ethidium bromide-stained 2% agarose gel .

A 10 μL aliquot of the PCR product (182 bp) was digested with "BciVI" restriction enzyme (NEB, UK) according to the manufacturer instructions and the restriction digest was separated in ethidium bromide-stained 2% agarose gel.

Table 1: The PCR-RFLP requirements for genotyping SNP rs76014067 T>C.

PCR primers	PCR product size	Restriction enzyme	Interpretation
F: CTCCCATTTCCACCCAGTCT	182 bp	BciVI	"C" allele : 182 bp (uncut)
R: CACAGACATCATGCCAGTGG			"T" allele : 93 + 89 bp

Statistical analysis

The genotypes and alleles frequencies in RPL patients and the controls were analyzed by standard odds ratio (OR) for risk of RPL at 95% confidence intervals (CI) and p values ≤ 0.05 were considered significant.

RESULTS

Genotype and allele distribution of the investigated polymorphism in RPL subjects and controls.

The genotype and allele frequencies of the study population are shown in (Table-2). Statistical analyses revealed that the "C/T" genotype and the "T" allele of rs760140467 polymorphism existed in significantly higher frequencies in the RPL cases.

Table 2. The frequencies of SNP rs760140467 genotypes and alleles in RPL and control groups

Genotype or allele	RPL group	Control group	OR (95% CI)	P value
C/C	74	90	3.16 (1.43-6.98)	0.0044*
C/T	26	10		
T/T	-	-		
"C" allele	174	190	2.84 (1.33-6.06)	0.0070*
"T" allele	26	10		

* Statistically significant

DISCUSSION

Successful implantation and maintenance of pregnancy relies on the immune tolerance imposed by the mother against her semi-allogeneic fetus. This immune tolerance is largely dictated by the structure of the microenvironment at the fetomaternal interface. Endometrial glycodeclin, the product of *PAEP* gene, is a major glycoprotein component of this microenvironment. Glycodeclin facilitates implantation and maintains pregnancy by inhibiting the immune response [10].

In the present work, we have for the first time demonstrated an association between RPL and the *PAEP* gene promoter SNP "rs760140467 C>T". We found that RPL risk in carriers of the "T" allele and the "C/T" genotype is higher than in women harboring the "C" allele or the "C/C" genotype (ORs = 2.84 and 3.16, respectively). The association between this polymorphism and RPL could be due to lower glycodeclin expression of the T-allele, as this polymorphism is located within a progesterone receptor binding site. Suppression of serum and endometrial glycodeclin levels has been shown to impair the preimplantation environment and to increase the rate of early pregnancy loss [11].

The association of this immune tolerance-related gene polymorphism may be useful for testing its relevance in other populations with different ethnic backgrounds and may assist in identifying treatments such as glycodeclin administration or enhancing glycodeclin gene expression. Meanwhile, research is ongoing to determine the importance of this polymorphism on a larger sample and to correlate the "T" allele with the level of glycodeclin in pregnant women.

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