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The HLA-G 14-bp Insertion/ Deletion Polymorphism in Recurrent Spontaneous Abortion among Iranian Women

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ABSTRACT

HLA-G is a non-classical HLA class Ib molecule with limited protein variability generated by alternative splicing. HLA-G displays immunotolerant properties and hence plays important roles in the maintenance of a successful pregnancy and maternal tolerance of the semiallogenic fetus. Polymorphism of the HLA-G gene may potentially affect the biological properties of the protein, and a 14-bp insertion/deletion polymorphism in exon 8 of the 3' untranslated region (3' UTR) of the HLA-G gene is thought to influence HLA-G expression.

To study the association of the 14-bp insertion/deletion (INDEL) polymorphism with the risk of recurrent spontaneous abortion (RSA), we used polymerase chain reaction (PCR) amplification, and genotyped 85 women in the case group (women who have had two or more unexplained RSA) and 85 women in the control group (women who have had at least one normal pregnancy). Our results showed that the frequencies of the−14 bp/−14 bp and +14 bp/+14 bp genotypes were reduced in women with RSA, while that of the +14 bp/−14 bp genotype was significantly increased in RSA compared with the control group of normal fertile women; no significant differences in the allele frequencies of the HLA-G 14-bp polymorphism were observed.

These results suggest a possible significance of the HLA-G 14-bp INDEL polymorphism in the outcome of pregnancy. However, further studies on other polymorphic sites in the 3 UTR and 5' UTR regions, as well as monitoring the serum HLA-G concentration are necessary in order to determine the potential implications of this marker in our population.

Keywords: Abortion; HLA-G; PCR, Polymorphism; 3' UTR

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INTRODUCTION

Early pregnancy loss is the most common complication of human gestation, occurring in as many

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as 75% of all women trying to conceive.[1] Approximately 5% of couples trying to conceive have suffered two successive miscarriages, and nearly 1%, three or more consecutive losses.[2-4] Human pregnancies are essentially never syngeneic;[5] therefore, specific mechanisms must exist for adjusting and moderating the maternal immune system so that the pregnant woman does not reject her own fetus.[6-8]

Human leukocyte antigens (HLA), as a major determinant of allograft rejection, have been extensively studied in the context of immune tolerance in pregnancy.[9,10] HLA-G is a non-classical human leukocyte antigen selectively expressed in fetal tissues at the maternal–fetal interface, and seems to be largely responsible for the reprogramming of the local maternal immune response. Such an expression pattern is unique among HLA genes, and indicates that HLA-G may be involved in interactions that are critical for the establishment and maintenance of pregnancy.[11-15] This function of HLA-G has been associated with its ability to inhibit cytotoxic T-lymphocyte and natural killer cell cytolytic functions,[16] which in turn is accomplished through interactions with the inhibitory receptors present on NK T, and antigen-presenting cells.[17-20]

The HLA-G gene is located on the short arm of chromosome 6 within the HLA region (6p21.2-21.3).[18,21] Seven HLA-G isoforms are generated by alternative splicing of the primary HLA-G transcript. Three of these isoforms (HLA-G5, -G6, and -G7) are soluble,[22] and abundantly found in the maternal circulation during pregnancy.[23] At least three polymorphic sites at the 3′ UTR have been associated with HLA-G mRNA regulation, including the 14-bp INDEL, +3142C-G, and +3187A-G.[24] There is a strong linkage disequilibrium (LD) among these three major 3′ UTR polymorphic sites.[25]

Harrison et al.[26] described the 14-bp INDEL polymorphism (5′-ATTTGTTCATGCCT-3′) located in the 3′ UTR region at position 3741 in exon 8 of the HLA-G gene (according to the reference sequence). Recent reports illustrate that it influences HLA-G mRNA stability and isoform splicing patterns, thereby modulating HLA-G expression levels.[6] A possible association has been found between this polymorphism and recurrent miscarriage[27,28] or the development of pregnancy, including increased birth weight and placental weight.[29] In the current study, we analyzed the occurrence of the 14-bp INDEL polymorphism in women with a history of two or more recurrent miscarriages, to investigate any correlation between this polymorphism and pregnancy failure, comparing with fertile women who have had at least one normal pregnancy as controls.

MATERIALS AND METHODS

Patients and Controls

Peripheral blood samples were obtained from 85 Iranian women who visited the Ebnesina Genetic Diagnostic Laboratory, Tabriz University of Medical Sciences, for evaluation of recurrent miscarriage, and from 85 healthy women who have had at least one normal pregnancy, as controls.

Informed consent was obtained from all the participants. Patients were enrolled in the study if they met the following criteria: suffered from two or more previous miscarriage, were aged less than 40 years, and had no identifiable causes, including anatomic, endocrinologic, cytogenetic, and autoimmune causes, for their history of recurrent miscarriage.[30] The routine screening and diagnostic protocol included cytogenetic studies in couples, hormonal measurements, assays for cardiolipin and lupus anticoagulant antibodies, antithrombin III, protein C, and protein S, as well as analysis of intrauterine contour by hysterosalpingography, sonohysterography, or hysteroscopy.

DNA Isolation

Genomic DNA was extracted from the peripheral blood samples using the salting-out procedure.[31]

HLA-G Typing

Exon 8 of the HLA-G gene was amplified using the primers GE14HLAG (5′-GTGATGGGCTGTTTAAAGTGTCACC-3′) and RHG4 (5′-GGAAGGAATGCAGTTCAGCATGA-3′).[17] The polymerase chain reaction (PCR) was carried out in a final reaction volume of 20 µl, which included 100 ng of genomic DNA, 10 pM of each primer, 1.5 U of Taq DNA polymerase, 2 µl of the 10× buffer, 1.5 mM of $MgCl_2$, and 0.2 mM of dNTPs. The reaction was performed in a thermal cycler (Eppendorf) with initial denaturation at 94 ˚C for 5 min, followed by 30 cycles at 94 ˚C for 30 s, 64 ˚C for 1 min, and 72 ˚C for 45 s, and a final extension at 72 ˚C for 10 min. The PCR products were analyzed by 8% non-denaturing polyacrylamide gel electrophoresis (200V for 80 min),

and the gels were stained using silver staining method to visualize the bands.

Statistical Analysis

The association of the HLA-G 14-bp INDEL polymorphism with RSA was examined using Chi-square test.

RESULTS

As illustrated in Figure 1, insertion or deletion of the 14-bp sequence in HLA-G generated PCR products of length 224 or 210 bp, respectively. Three different genotypes (+14 bp/+14 bp, +14 bp/−14 bp, and −14 bp/−14 bp) were distinguishable by 8% non-denaturing polyacrylamide gel electrophoresis.

Our results showed that the frequencies of the homozygous genotypes (−14 bp/−14 bp and +14 bp/+14 bp) were reduced in women with recurrent miscarriage (Table 1). However, the frequency of the +14 bp/−14 bp heterozygous genotype was significantly increased in women with recurrent miscarriages compared to the normal fertile controls (χ^2= 2.79, p=0.59). There were no significant differences in allele frequencies of the 14-bp polymorphism between controls and RSA women (χ^2 = 54.16, p=0.0019).

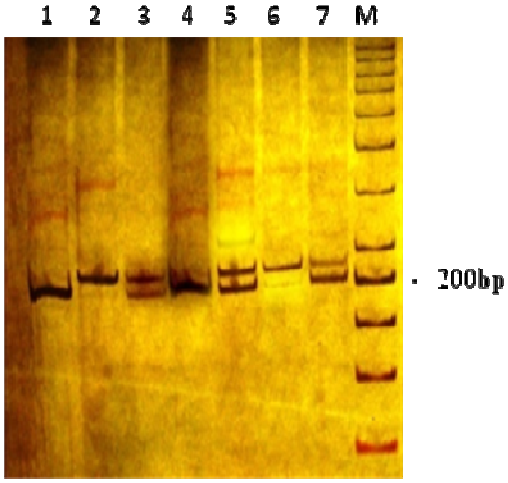

Figure 1. Detection of the 14-bp insertion/deletion polymorphism with polyacrylamide gel electrophoresis. Lane M, 50bp ladder (Fermentas); lanes 3, 5, 7 heterozygote; lanes 2, 6 homozygote for insertion; lanes 1, 4 homozygote for deletion.

Table 1. Frequencies of the 14 bp INDEL genotype/allele in exon 8 of HLA-G gene in control and RSA groups

	Fertile controls, n (%)	RSA group, n (%)
1) Genotype		
-14bp/-14bp	16 (18.8)	8 (9.4)
+14bp/-14bp	55 (64.7)	74 (87.1)
+14bp/+14bp	14 (16.5)	3 (3.5)
N	85	85
2) Allele		
14-bp insertion	83 (48.8)	80 (47.1)
14-bp deletion	87 (51.2)	90 (52.9)
N	170	170

RSA, recurrent spontaneous abortion.

1) χ^2 = 2.79, p = 0.59.

2) χ^2 = 54.16, p = 0.0019.

DISCUSSION

Human and nonhuman primate pregnancies provide natural models for studying the mechanisms of immune tolerance and the features of immune privileged sites. Studies on pregnancy over the past half-century have provided immunologists with definitive proof that in successful transplants, which include the fetus, “foreign” tissue mediates various overlapping and complementary mechanisms to avoid rejection.

Of these, selection of HLA-G, with a gene with limited polymorphism, for expression at the maternal–fetal interface is of major importance.[18] HLA-G belongs to a family of immunomodulatory glycoproteins, and alternative splicing of the gene’s single message results in multiple isoforms.

While HLA-G is important for immune tolerance during pregnancy, the role of HLA-G polymorphisms in governing pregnancy outcome remains to be addressed. The hypotheses regarding the functions of this polymorphism include the following:

1) During mRNA processing, this sequence may function as a cryptic branch point and induce alternative splicing of the HLA-G mRNA.[6]

2) Stability of the mRNA may be influenced by the AU-rich element of 3′ UTR, because the initial pentameric sequence (AUUUG) of the 14-bp polymorphic region has an AU pentamer-like effect, and may be involved in deadenylation and subsequent decay of the mRNA.[32]

3) The dominant effect of low secretors (+14 bp)

over the high secretors (−14 bp) in the heterozygote decreases the amount of soluble HLA-G.[17,33,34]

4) According to basic research, the 14-bp sequence was considered to be a target for specific microRNAs, as this sequence remains in an open configuration after mRNA folding. Modifications of mRNA secondary structure due to its deletion may thereby influence mRNA stability and microRNA accessibility.[35-37]

In the current study, we focused on the 14-bp INDEL polymorphism in the 3′ UTR region in exon 8 of the HLA-G gene. Our results revealed an increased frequency of the heterozygous genotype in women with recurrent miscarriage, compared to the control normal fertile women (Table 1). No significant differences in allele frequencies of the 14-bp polymorphism were detectable between these groups.

In comparison, several studies have reported more homozygotes for the 14-bp insertion sequence in RSA compared to normal fertile women, although in some studies this correlation was not noteworthy.[27,38-41] Other studies found an increased number of heterozygous individuals among women with RSA,[42,43] and yet others have reported no correlation at all.[44,45] In addition, the latter study by Svrlkmpvrtdms[45] reported an increased frequency of individuals in the RSA group carrying both the 14-bp insertion and a novel SNP in exon 8, a T-C mutation at position 1570.

While considering the impact of the findings in the present study, important limitations must also be regarded. First, this study was a single-center case-controlled study, and subsequent investigations employing a larger number of patients would be useful for confirming the role of this polymorphism in governing RSA risk. Secondly, the link between the HLA-G 14-bp INDEL polymorphism and RSA was observed among subjects from the northwest of Iran. Further studies are required to clarify this association among populations in different regions of Iran.

Controversial results have been obtained in various studies with respect to the insertion/deletion polymorphism. These varied results were perhaps caused by differences in the distribution of the polymorphism due to ethnicity of the groups under study, and possible linkage disequilibrium with other HLA variants. The polymorphic sites at the 3′ UTR seem to be organized into several haplotypes in different populations, each of them associated with either a single or a group of coding and promoter region polymorphisms, creating extended HLA-G haplotypes that result in altered patterns of HLA-G mRNA (and thereby protein) isoforms and their concentration.[17,46] On the other hand, some studies suggest that both HLA-G and killer inhibitory receptors (KIR) polymorphisms, as well as other polymorphic genes in the HLA family, may contribute to these variations, which also increases the complexity of the present study.[47,48]

According to recent studies, the presence of the 14-bp insertion has been correlated with reduced levels of HLA-G mRNA, possibly providing an explanation for its increased frequency among women with RSA.[47,49] Taken together, the studies mentioned previously confirm that higher serum HLA-G levels are associated with lower RSA risk.[27,41,49]

In the light of several recent studies, it is now known that reduced or aberrant HLA-G expression may be associated with certain diseases, such as multiple sclerosis,[50] breast cancer,[51] and Crohn's disease,[52] which can be linked to HLA-G polymorphism.

Based on these observations, it can be explicated that HLA-G as a single factor has a very modest effect with respect to the risk for recurrent miscarriage. Although the 14-bp INDEL polymorphism is responsible for the post-transcriptional regulation of the HLA-G gene, further studies addressing the other polymorphic sites located at the 5′ UTR that is close to the transcription factor-binding sites or at the 3′ UTR that influences HLA-G mRNA accessibility are required.

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