

Role of 14-bp deletion/insertion polymorphism in exon 8 of the HLA-G gene in recurrent spontaneous abortion patients

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ABSTRACT

BACKGROUND: Human leukocyte antigen (HLA)-G belongs to the nonclassical Class I major histocompatibility complex, and is predominantly and specifically found on the extravillous cytotrophoblast cells of the placenta. HLA-G has been postulated as an important immunotolerant molecule in maintaining successful pregnancy and maternal tolerance of the semiallogenic fetus. Recent reports indicate that the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene influences the HLA-G mRNA stability and isoform splicing patterns, thus modulating the levels of HLA-G expression. **AIM:** The aim was to study the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene. **MATERIALS AND METHODS:** A total of 50 women with unexplained three or more recurrent spontaneous abortions (RSAs) and 41 normal healthy control women who have had normal pregnancies and were genotyped for the 14-bp deletion/insertion polymorphism were genotyped for the 14-bp deletion/insertion polymorphism by polymerase chain reaction for exon 8-specific primers. **RESULTS:** It was found that the 14-bp allele deletion frequency was lower in patients (67%) versus controls (73%), while 14-bp allele insertion was higher among patients (33%) versus controls (9%). Similarly, the homozygous deletion haplotype was higher among the controls (80.48%); the heterozygous insertion deletion haplotype (34%) and homozygous insertion haplotype (16%) were higher in RSA patients. The HLA haplotype HLA A*02:11_B*40:06:01:01 was increased among RSA women compared to controls. **CONCLUSION:** Our results suggest that 14-bp deletion/insertion polymorphisms might have importance in the outcome of pregnancy and the 14-bp deletion polymorphism in exon 8 of the HLA-G gene may be important from an evolutionary perspective of successful pregnancy.

KEY WORDS: HLA-G 14-bp deletion/insertion gene, India, RSA

INTRODUCTION

The success of pregnancy where the semiallogenic foreign graft (fetus) resides comfortably within the mother's uterus during the 9 months without eliciting an immune response against it defies the concepts of immunology. During pregnancy, the maternal immune system is in close contact with the cells of the semiallogenic fetus. Hence, there must exist specific mechanisms that would prevent the mother to reject the fetus and the mechanism which when aberrated might give rise to complications in pregnancy. The so called nonclassical Class I human leukocyte antigen (HLA)-G may be the answer to this

riddle.^[1] HLA-G has been specifically and predominantly found on the cytotrophoblast cells and has been known to be involved in the maternofetal immunotolerance.^[2] The trophoblast cells which are of the fetal origin do not express Class I and Class II HLA antigens except for a weak expression of HLA-C. HLA-G belongs to the nonclassical Class I major histocompatibility complex (MHC), and is predominantly and specifically found on the extravillous cytotrophoblast cells of the placenta. HLA-G has been postulated as an important immunotolerant molecule in maintaining successful pregnancy and maternal tolerance of the semiallogenic fetus. Reports indicate that the 14-bp

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deletion/insertion polymorphism in exon 8 of the 3'UTR region of the HLA-G gene influences the HLA-G mRNA stability and isoform splicing patterns, thus modulating the levels of HLA-G expression. Although it is structurally similar to the Class I MHC proteins, it exhibits certain unique characteristic features. One remarkable difference between the HLA Class I and II genes is that the latter show limited polymorphism. Furthermore, in context of HLA-G, seven alternatively spliced transcripts have been identified, of which four encode membrane-bound and three encode soluble proteins.^[3] Also the expression of Class I antigens is ubiquitous whereas expression of Class II antigens may be tissue/organ specific and/or conditional.^[4,5]

Harrison *et al.*^[6] were the first to describe a 14-bp deletion/insertion polymorphism (5'-ATTTGTTTCATGCCT-3') in the 3'UTR region of the HLA-G gene located at position 3741 in exon 8 (according to the reference sequence).^[7] The various polymorphisms in the HLA-G gene might vary the mRNA profile expression and also the type of protein formed. HLA-G features the low level of allelic polymorphisms with only 46 alleles identified to date (16 based on exon and 7 based on intron polymorphisms) and encodes 7 protein isoforms generated by alternative splicing.^[8]

HLA-G exerts its inhibitory functions on cell cytotoxicity^[9] and alloproliferative responses through interaction with at least three inhibitory receptors present on NK, T, and antigen-presenting cells, namely, LILRB1 (leukocyte immunoglobulin-like receptor, subfamily B, with TM and ITIM domains, member 1; ILT-2/CD85j), LILRB2 (leukocyte immunoglobulin-like receptor, subfamily B, with TM and ITIM domains, member 2; ILT-4/CD85d), and KIR2DL4 (killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, member 4; CD158d).^[11] Membrane-associated and/or soluble HLA-G may play a critical role in regulating CD8+ T cells during pregnancy by eliminating alloreactive (ant paternal) T cells. It has been seen that HLA-G induces CD8+ T-cell apoptosis through the Fas/FasL pathway.^[10]

Studies on HLA-G polymorphisms in recurrent spontaneous abortions (RSAs) have revealed an association of different HLA-G alleles with RSAs.^[11-17] An association between *G0105N that impairs the production of the G1 isoform in RSA women has been shown in various cases.^[11] The deletion of C from codon 130 results in a frameshift in the G*0105N allele. HLA-G allelic variants are associated with varying mRNA levels of different isoforms.^[12]

MATERIALS AND METHODS

Samples and genomic DNA extraction

A total of 50 women with unexplained three or more RSAs and 41 normal healthy control women who have

had minimum one or more normal pregnancies were genotyped. A routine investigation for antinuclear antibodies (ANAs), auto-antibodies against dsDNA, antineutrophil cytoplasmic antibodies (ANCA), anticardiolipin antibodies (ACLAs) and Lupus-like anticoagulants (LAs) and chromosomal abnormalities in women who had APLA or hereditary thrombophilia, chromosomal abnormalities in either of the couple to increase the risk of losses, thyroid function, diabetes or deranged OGGT, abnormalities of the uterus such as septum were all excluded in our study. All other causes such as structural, infective, hormonal, and hereditary thrombophilias were ruled out among our patients. ANAs, auto-antibodies against dsDNA, ANCA, ACLAs, and LAs and chromosomal abnormalities were done. Five milliliters of blood was taken from both controls and RSA women and was collected in EDTA vials. Genomic DNA was extracted from the peripheral blood by the salting out procedure.^[18]

For the polymerase chain reaction (PCR) amplification of exon 8 of the HLA-G gene, exon 8 was amplified using primers GE14HLA G: 5'-GTGATGGGCTGTTTAAAGTGCACC-3', and RHG4: 5'-GGAAGGAATGCAGTTCAGCATGA-3'.^[9] Genotyping for the 14-bp deletion polymorphism was done by electrophoresis. The PCR products were run on a 12% nondenaturing polyacrylamide gel and stained with ethidium bromide. The amplified PCR products were either of 224 bp or 210 bp depending on the deletion of 14 bp from exon 8. The sizing of the PCR products was done using pBR322/HaeIII marker DNA; the PCR product was visualized and scored by two different observers. Differences between the groups were analyzed using graphpad software.

RESULTS

Figure 1 shows the gel photograph of HLA-G 14-bp exon 8-specific amplified products. The frequency of deletion was 89.02% in controls and 67% in RSA patients. The frequency of insertion was 10.97% in controls and 33% in RSA patients [Table 1]. The statistical analysis of the results are presented in Table 2.

DISCUSSION

HLA-G is the predominant HLA antigen found specifically on the extravillous cytotrophoblast cells of the placenta and thus is proposed to have a major role in the maternal acceptance of the fetus during pregnancy. This is accomplished by downregulating the maternal NK cells, thus avoiding an innate immune response and also downregulating the T cells thus leading to maternofetal tolerance. Also, it is seen that HLA-G can bind peptides^[14] and may be responsible for the elimination of viral infection in the developing fetus.

Our study indicates that there were more homozygotes for the 14-bp insertion found in RSA women as compared to normals and more heterozygote +14 bp/-14 bp women were found in the RSA group as compared to the normal fertile females. Furthermore, the +14 bp/+14 bp genotype was found in RSA patients with abortions greater than 3 as observed by Abbas *et al.*,^[11] Tripathi *et al.*,^[12] and Yan *et al.*^[13] They showed an increase in the frequency of 14-bp insertion alleles in the RSA group as compared to controls whereas in contrast, Hviid *et al.*^[19] observed more number of heterozygotes in controls. Comparing our results of the maternal 14-bp genotype with those from different ethnic populations all over the world, it was observed that normal

fertile women of Western India show a considerably higher percentage of the 14-bp homozygous deletion as compared to other ethnicities. The number of heterozygotes found in our controls is lesser as compared to others. This discrepancy might be due to the ethnic variations.

The exact biological role of the 14-bp deletion is yet not exactly elucidated and the results of the study on the 14-bp deletion polymorphism in the maintenance of pregnancy are very controversial. However, it has been seen that there may be a direct association between the 14-bp sequence and the altered pattern in the HLA-G mRNA (and thereby protein) isoform and its concentration.^[20] It has been reported that the HLA-G alleles are all between high secretors and low secretors, the alleles G*01013 (+14 bp) and G*0105N (+14 bp) being low secretors, G*01041 (-14 bp) a high secretor, and G*01011 and G*01012 being between the two levels.^[13-21] Furthermore, G*01041 (-14 bp) was shown to be associated with a high plasma level of sHLA-G. It has been suggested that the low secretor (one with +14 bp) was dominant over the high secretor (one with -14 bp) allele in the heterozygous condition. This dominant effect of the low secretors over the high secretors decreases the amount of sHLA-G. In this study, we have found a higher occurrence of -14 bp/-14 bp in controls compared to RSA patients. The percentage of heterozygotes was high among RSA patients comparatively. Probably, the introduction of the 14-bp allele dominated the high secretor effect of the -14-bp allele in heterozygotes and further may decrease the amount of HLA-G secreted. As decreased soluble HLA-G is associated with a poor outcome in pregnancy, this favors more number of heterozygotes in RSA women as observed. Soluble HLA-G may dramatically influence both maternal and fetal immune responses and thus decrease or prevent the maternal T-cell alloimmune attack on the fetus. However, further studies are required to elucidate the exact biological mechanism behind the dominant effect of the low secretor allele over high secretor HLA-G alleles.

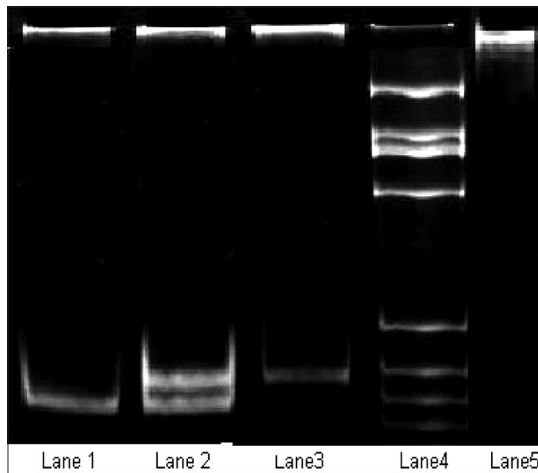


Figure 1: SDS-PAGE of the 14-bp amplified product. Lane 1: -14 bp/-14 bp (210 bp); lane 2: +14 bp/-14 bp; lane 3: +14 bp/+14 bp; lane 4: pBR322/HaeIII marker; lane 5: -ve control lane

Table 1: Frequencies of the 14 bp insertion/deletion genotype and allele distribution in RSA and control women

Genotype	RSA patients (%) N=50	Controls (%) N=41
del/del	50	80.48
ins/ins	16	2.4
Heterozygous	34	17
Allele		
14-bp insertion	33	10.97
14-bp deletion	67	89.02

RSA: Recurrent spontaneous abortion

CONCLUSION

Our results indicate that 14-bp deletion/insertion polymorphisms have importance in the outcome of

Table 2: Distribution of the 14-bp insertion/deletion allele and genotype in RSA and control women

Genotype	Patients N=50 %	Controls N=41 %	OR	χ ² -value	CI	EF	PF	P value
-14 bp/-14 bp	50	80.48	0.242	7.788	0.09-0.62		0.61	0.0053**
-14 bp/+14 bp	34	17	2.502	2.509	0.91-6.81			0.11
+14 bp/+14 bp	16	2.4	7.619	3.251	0.91-63.73	0.138		0.0714
Allele	N=100 %AF	N=82 %AF						
-14 bp	67	89.02	0.25	11.102	0.11-0.56		0.667	0.0009**
+14 bp	33	10.97	3.995	11.102	1.78-8.96	0.247		0.0009**

%AF - Allele frequency in percentage; CI - Confidence interval; EF - Etiological fraction; OR - Odds ratio; PF - Preventive fraction. **Significant P-value; RSA: Recurrent spontaneous abortion

pregnancy and the 14-bp deletion polymorphism in exon 8 of the HLA-G gene may be important from an evolutionary perspective of successful pregnancy. However, more genotyping investigations and functional studies on the expression patterns and immune regulation are essential to elucidate the role of HLA-G in pregnancy.

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