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Review Article

Relation Between *HLA-G* Gene Null Allele (*HLA-G*0105N*) and Recurrent Miscarriage

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Human leukocyte antigen-G (HLA-G) is a nonclassical HLA-class I antigen located on chromosome 6. HLA-G is highly expressed on cytotrophoblast cells at the fetomaternal interface and involved in the development of pregnant uterus as an immune privileged site. Expression of HLA-G is thought to have a critical role in the protection of the semiallogenic fetus from maternal immune attack during pregnancy. HLA-G molecules bind inhibitory receptors on maternal T cells and NK cells and subsequently inhibit their cytolytic activities. Because of mRNA alternative splicing of *HLA-G* primary transcript, the HLA-G protein exists in both membrane-bound (HLA-G1 to G4) and soluble (HLA-G5 to G7) isoforms. *HLA-G* gene contains 15 alleles, including the *HLA-G*0105N* null allele. A single base-pair deletion of a cytosine (1597delC) results in open reading frame mutation, which leads to a premature stop codon. The *HLA-G*0105N* allele is unable to generate the HLA-G1, HLA-G5, and HLA-G4 isoforms. However, it is still able to produce other HLA-G proteins, in which exon 3 is removed by alternative splicing, including HLA-G2, G3, G6 and G7 isoforms. *HLA-G*0105N* null allele has been described in healthy adults with successful and normal pregnancies, which suggests that HLA-G function is not restricted to the HLA-G1 isoform. Description of healthy individual homozygous for *HLA-G*0105N* allele recommends that truncated HLA-G2 and G3 isoforms encoded by null allele are able to compensate for the lack of the HLA-G1, G4 and G5 isoforms. Results of the numerous studies on the null allele of *HLA-G* gene indicated that its selection may have increased the frequency of the *HLA-G*0105N*. Studies on the null allele of *HLA-G* gene could be useful in determining the frequency of genetic variants of *HLA-G* alleles in different ethnic groups.

Keywords: HLA-G Antigens; Abortion, Habitual; HLA-G*01:05N Antigen

The human major histocompatibility complex (MHC) genes are clustered on the short arm of chromosome 6 and divided into class I (class Ia: HLA-A, B, and C; class Ib: HLA-E, G and F) and class II (HLA-DR, DQ and DP) genes. Human leukocyte antigen-G (HLA-G) is a non-classical HLA-class I antigen located on chromosome 6p21 at the telomeric end of the MHC region, close to HLA-A (1). Contrary to the classical HLA class I genes that have most polymorphic regions with so many alleles in the human genome, *HLA-G* is distinguished by low numbers of alleles, i.e., with ~20 nucleotide alleles encoding less than 10 different protein sequences (2). *HLA-G* is highly expressed on invasive extravillous cytotrophoblasts at the fetomaternal interface. Expression of *HLA-G* is thought to have a critical role in the protection of the semiallogenic fetus from maternal immune recognition during pregnancy (3, 4). *HLA-G* is involved in development of pregnant uterus as an immune privileged site (5). Therefore, it has been proposed that the reduced *HLA-G* gene transcription and translation, and subsequently diminished placental HLA-G expression is associated with complications of pregnancy such as preeclampsia, recurrent miscarriage, and implantation failure in IVF (In vitro fertilisation) (6, 7).

The main function of HLA-G is not antigen presentation; it plays a significant role as an immune tolerogenic molecule that may also protect the semiallograft fetus from maternal immune system as well as tumor escape and other types of allogenic tissue acceptance (8). In vivo biological relevance of HLA-G protein expression on cytotrophoblasts is somehow essential for successful pregnancy and any graft acceptance (5). Secretion of soluble HLA-G could lead to success in IVF treatments (effective fertilization or intracytoplasmic sperm injection). Therefore, HLA-G protein expression on fetal-maternal interface was suggested as contributing to the immune-privileged sites for the fetus during 9 months (9). Both membrane-bound and soluble HLA-G molecules as an inhibitory ligand bind to inhibitory receptors of maternal natural killer cells (NK) and cytotoxic T-lymphocytes (CTL), thus inhibit allogenic maternal responses such as proliferative T cell

response, antigen-specific T-cell cytotoxicity, and NK cell-mediated cytolysis. Additionally, soluble HLA-G isoforms are able to induce apoptosis of both activated CTL and NK cells (10, 11). This inhibition is mediated through direct interaction with inhibitory receptors such as leukocyte Ig-like receptor 1 and 2 (LIR-1 and LIR-2), killer Ig-like receptor 2 (KIR2 DL4) or p49 (12, 13). The signal peptide of HLA-G permits stable expression and up-regulation of HLA-E on trophoblasts surface (14). HLA-E is another nonclassical HLA class I molecule and the most important ligand for the inhibition of NK cells. 1 HLA-E helps the fetus to evade maternal immune response, perhaps by an indirect inhibitory pathway and blocking of CD94/NKG2A1, NK-cell inhibitory receptor (15).

HLA-G, like other class I genes, has 8 exons: exon 1 encodes a signal peptide, exons 2, 3, and 4, encode extracellular globular domains (α1, α2 and α3 domains, respectively), exon 5 encodes membrane-anchored domain and exons 6 and 7 encode one intracytoplasmic domain (16). α1 and α2 domains create the peptide-binding groove and α3 domain is the binding site for β2 microglobuline and leukocyte Ig-like receptor 1 and 2 (LIR-1 and LIR-2), which are inhibitory receptors. Unlike the polymorphism in classical HLA class I molecules, which is concentrated nearby the peptide binding cleft, the limited polymorphism of HLA-G is located around α1, α2, and α3 domains (17). Because of mRNA alternative splicing of HLA-G primary transcript, the HLA-G protein exists in both soluble and membrane-bound isoforms. These include 7 proteins, 4 membrane-bound isoforms (HLA-G1, G2, G3, and G4) and 3 soluble isoforms (HLA-G5, G6, and G7) (18, 19). Full-length HLA-G mRNA encodes the HLA-G1 protein, which has classical HLA class I structure (3 extracellular domains, 1 encodes transmembrane domain and 1 intracellular domain) and is associated with β2-microglobuline. *HLA-G1* is structurally similar to other class I genes, except for its shortened cytoplasmic tail. A stop codon in exon 6 causes a truncated cytoplasmic tail that results in the elongated expression of HLA-G at the cell surface (1). HLA-G2, G3, and G4 isoforms result from the removal of exon 3 (α2 domain), exons 3, and 4 (α 2 and α3 domains), and exon 4 (α3 domain) from the primary transcript, respectively. Inclusion of intron 2 or 4 in the mature mRNA may generate premature stop codons, which result in the absence of transmembrane domains and, as a consequence, leads to generate soluble isoforms of HLA-G molecule with an additional 21 amino acids after the α3 domain. Soluble isoforms comprise HLA-G5 (the soluble isoform of full-length HLA-G1), HLA-G6 (the soluble isoform of HLA-G2), and HLA G7 (the soluble isoform of HLA-G3) (17, 18).

HLA-G gene contains 15 alleles, including the *HLA-G*0105N* null allele. A single base-pair deletion of a cytosine at codon 130 (1597delC) in exon 3 (encoding the α2 domain) results in a gap in the open reading frame leading to a premature stop codon at the beginning of exon 4 (encoding the α3 domain) (20). *HLA-G*0105N* allele was named "null" due to its incapability to generate the full length membrane-bound HLA-G1, well known as the functional HLA-G protein, and full length soluble isoforms HLA-G5 as well as the spliced HLA-G4 isoform. However, *HLAG*0105N* is still able to produce other HLA-G proteins such as the membrane-bound HLA-G2 and G3 isoforms and the soluble HLA-G6 and G7 isoforms, in all of them disrupted exon 3 (encoding α2 domain) is removed by alternative splicing. HLA-G function is not restricted to the HLA-G1 isoform; HLA-G2, -G3, -G6 and -G7 isoforms are functional HLA-G proteins with the ability to inhibit NK cell cytolysis and may substitute for HLA-G1 and -G5 in immune tolerogenic functions (21). *HLA-G*0105N* null allele has been described in healthy adults with successful and normal pregnancies. Description of healthy individual homozygous for *HLA-G*0105N* allele recommends that any of the other truncated HLA-G isoforms (or at least HLA-G2 and -G3) encoded by null allele may possess the protective functions of HLA-G and able to compensate for the lack of the HLA-G1, G4, and G5 isoforms (22, 23). These functional truncated HLA-G molecules , like the full-length HLA-G1, act as inhibitory molecules toward maternal NK cells (innate effectors), CTLs (CTL effectors), and play a role in maintaining the immune privileged status of the fetal-maternal tolerance, in the absence of the HLA-G1, G5, and G4 isoforms (21, 24). After blockage of HLA-G1 using specific monoclonal antibody (MEM-G/04), other HLA-G proteins, which are produced by HLA-G transfected cells can protect the fetus from maternal NK cell attacks and contribute to immune privilege for the fetus. Because of the absence of α2 or α3 domains in HLA-G3 isoform, probably the α1 domain, which is shared with all HLA-G isoforms, may have the functional region. HLA-G2 and G3 isoforms are expressed on trophoblastic cells obtained from first-trimester of normal pregnancies (21). Staining of homozygous *HLA-G*0105N* placentas by a monoclonal antibody specific for soluble HLA-G proteins, indicated that trophoblasts of these individuals may express the HLA-G6 (soluble HLAG2-like isoform) and HLA-G7 (soluble HLAG3-like isoform). Besides, soluble isoforms of HLA-G, HLA-G6, and HLA-G7 circulate in maternal blood, which supported the idea of implication of these proteins in fetomaternal tolerance (25). Results of the numerous studies on the null allele of *HLA-G* gene indicated that selection may have increased the frequency of *HLA-G*0105N* (26). Studies on the null allele of *HLA-G* gene could be useful in determining the frequency of genetic variants of *HLA-G* alleles in different ethnic populations. *HLA-G*0105N* null allele has been found in a variety of ethnic populations with frequencies of 9% in Iranians, 8% in African Americans, 4.8% in Ghanaians, 3% in Spaniards, 2.3% in mixed German-Croatians, 0.6% in Danish population and 11% in the Shona tribe of Zimbabwe. This deletion was not found in American Caucasian populations or Japanese (27-31).

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