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Null Allele Frequencies at HLA-G Locus in Iranian Healthy Subjects

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ABSTRACT

Background: HLA-G gene contains 15 alleles including a null allele, HLA-G*0105N. Previous studies have shown that HLA-G*0105N does not encode the complete HLA-G1 or HLA-G5 isoforms but encodes a functional HLA-G protein with the ability to inhibit NK cell cytolysis. Thus, although the biological functions of HLA-G1 and HLA-G5 proteins are abrogated, other isoforms such as HLA-G2 can replace their roles. Studies on the null allele of HLA-G gene could be useful in understanding the genetic variants of HLA-G alleles in ethnic groups. **Objective:** The goal of this research was to determine the frequency of HLA-G*0105N null allele in Iranian healthy subjects. **Methods:** The frequency of HLA-G*0105N null allele was evaluated in Iranian healthy subjects by PCR-RFLP method. Genomic DNA was isolated from the whole blood of 100 randomly selected, healthy, unrelated Iranian individuals using salting-out technique followed by PCR amplification of the exon 3 of HLA-G gene. PCR products were digested with PpUM-1 and the resulted fragments were analyzed using gel electrophoresis. **Results:** In this study the restriction enzyme digestion confirmed homozygous HLA-G*0105N null allele for 9 % of the population. Furthermore obtained results indicated that the total frequency of HLA-G*0105N null allele was 20 % in the studied population of Iran. **Conclusion:** The final data analysis showed that the total frequency of this allele in Iranian people was higher than other ethnic groups that have been studied so far.

Keywords: Frequency, HLA-G*0105N antigen, Iran, RFLPs

INTRODUCTION

Human leukocyte antigen HLA-G is a non-classical HLA-class I antigen, located within the major histocompatibility complex (MHC) at 6p21.3, one of the most polymorphic regions in the human genome (1). In contrast to classical HLA class I genes that are highly polymorphic (2), HLA-G shows only minimal variation, with ~20 nucleotide alleles encoding less than 10 different protein sequences. Unlike the polymorphism in

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classical HLA class I molecules, which is concentrated around the peptide binding groove, the limited polymorphism of HLA-G is located between the α1, α2 and α3 domains (3). HLA-G is mainly expressed on invasive trophoblastic cells and is thought to be a mediator of maternal–fetal tolerance. HLA-G molecules bind inhibitory receptors on maternal T lymphocytes and NK cells and thereby, block their cytolytic activities and protect the fetus from the attack of maternal immune system. Expression of HLA-G is also associated with tumor escape and favoring graft acceptance (4). HLA-G, interacting with NK cells, can present nonamer peptides and bind CD8 in an analogous way to a classical HLA class I. The HLA-G protein exists in soluble and membrane-bound isoforms produced through alternative splicing. These include seven proteins, four membrane bound (HLA-G1 through –G4) and three soluble (HLA-G5 through -G7) (1,4). The HLA-G gene consists of 15 alleles, including the HLA-G*0105N null allele, which is characterized by a single base-pair deletion in exon 3 (5). This deletion of a single cytosine at codon 130 results in a gap in the open reading frame, causing a premature stop near the beginning of exon 4, which blocks translation of HLA-G1 and -G5. However, HLA-G*0105N is able to maintain translation of both the membrane-bound HLA-G2 and -G3 proteins and the soluble HLA-G6 and -G7 proteins. HLA-G*0105N null allele has been described in healthy adults with normal gestations and deliveries (without complications). The detection of normal individuals who are genetically homozygous for HLA-G*0105N allele suggests that the HLA-G isoforms encoded by this allele possess the functions capable of compensating for the absence of both the HLA-G1 and -G5 proteins and maintaining the immune privileged status of the fetal-maternal interface (6,7).
Based on the results of the numerous studies on the HLA-G*0105N allele frequency in different ethnic groups, and those of the previously mentioned observations, that selection may have increased the frequency of this allele (8), we evaluated its frequency in Iranian healthy subjects and compared it with other ethnic populations.

MATERIALS AND METHODS

Population. In this study, 100 samples from healthy unrelated Iranian individuals, as donor candidates for Bone Marrow Transplantation (BMT), referred to Iranian Blood Transfusion Organization (Tehran, Iran) were randomly selected.
Cell Line. JEG-3 (a human choriocarcinoma) cell line was obtained from the National Cell Bank of the Pasteur Institute of Iran. The cell line was used as a positive control because of its continuous expression of HLA-G. The JEG-3 cells were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.
DNA Extraction. DNA was extracted and purified from the whole blood samples collected in 4.5 mM EDTA using salting-out technique (9). The concentration, purity and integrity of DNA were assessed by biophotometer and electrophoresis on 2% agarose gel.
PCR-RFLP Analysis. HLA-G typing was carried out with polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP).
PCR was carried out with 500 ng of genomic DNA in a total volume of 25 μL of the reaction mixture containing: 0.4 μM of the primers (forward: 5′-CAC ACC CTC CAG TGG ATG AT-3′) and (reverse: 5′-GGT ACC CGC GCG CTG CAG CA-3'), 1.1x reaction buffer (Roche), 4.5 mM MgCL2, 250 μM dNTPs and 2.5 U of Taq DNA polymerase (Genet Bio).

The following PCR conditions were used: initial temperature denaturation at 94º C for 2 min, followed by 10 cycles of 94º C for 15 s, 63º C for 30s, and 68º C for 1 min and another 29 cycles of 94º C for 15 s, 54.7 º C for 30s and 68º C for 1 min. The program was followed by a final extension step at 72º C for 8 min. The restriction fragment length polymorphism was carried out by overnight digestion with 1 U of PpuM-I enzyme (Fermentas, Lithuania), 2 µl of 10x buffer Tango, 10 µl of PCR product and 8 µl of dH2O. The restriction digestion was analyzed using electrophoresis on a 2.5% agarose gel and staining with ethidium bromide. Absence of the restriction site for the PpuM-I enzyme in the exon 3 of HLA-G was illustrated for the presence of HLA-G*0105N allele (10).

RESULTS

The results of PCR-RFLP method are exemplified in Figures 1 and 2. HLA-G*0105N allele was distinguished according to the absence of the restriction site for the PpuM-I enzyme. The results revealed that no HLA-G*0105N null allele is detected in the cultured Jeg-3 control cells (Figure 2).

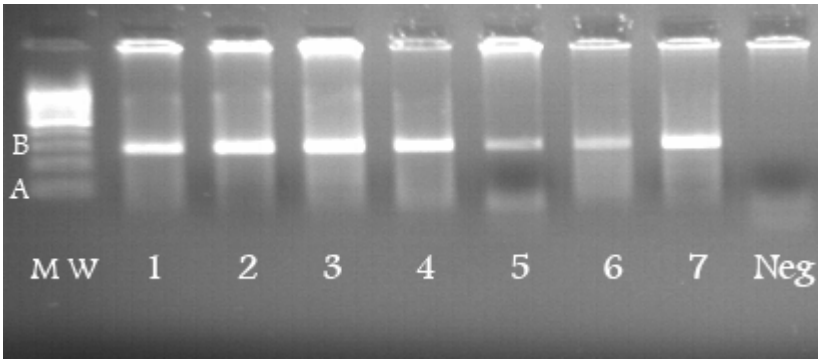

Figure 1. The amplified exon 3 gene fragment produced in the PCR procedure. The lanes represented: MW: the 100 bp DNA ladder (A: 100 and B: 300 bp bands of the marker), Lanes 1-7: Amplified HLA-G exon 3 fragments (276 bp), Neg: the negative control.

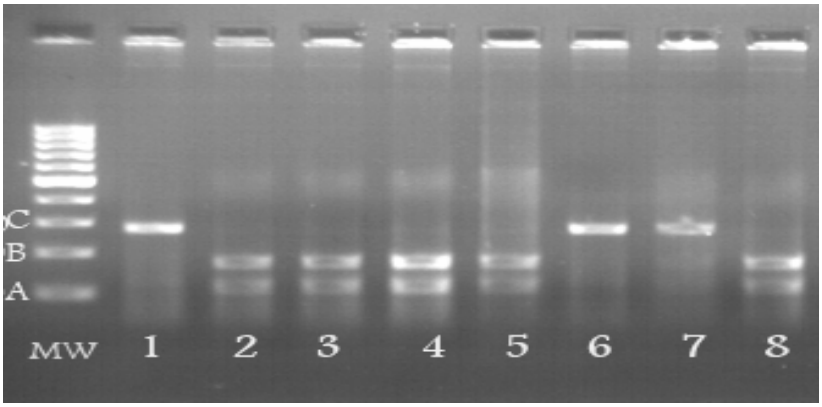

Figure 2. PCR-RFLP analysis of HLA-G*0105N null allele in the studied healthy individuals. The PCR products were digested overnight with restriction enzyme PpUM-1. The lanes represented: MW: the 100 bp DNA ladder (A: 100, B: 200 and C: 300 bp bands of the marker), and subsequently: Lane 1: Amplified HLA-G exon 3 before restriction digestion (276 bp), Lane 2: PpUM-1 digested fragments of HLA-G exon 3 in JEG-3 cell line and Lanes 3-5 and 8: the digested frag-

ments (168 and 108 bp) after restriction digestion in the normal samples. HLA-G*0105N allele was detected in the samples of lanes 6 and 7.

Our finding in the present study using PCR method and a specific restriction site of HLA-G gene in exon 3, demonstrated that the frequency of HLA-G*0105N null allele is present in Iranian population. Nine individuals were found to be homozygous and 22 heterozygous for this gene. It could be estimated that the total frequency of this allele was 20 % in the studied population of Iran.

DISCUSSION

The role of HLA-G gene has been proposed in cancer and pregnancy outcome (11-13). It consists of 15 alleles, including a null allele, HLA-G*0105N. HLA-G*0105N is a null allele arising from a single nucleotide deletion and frame shift that introduces a premature stop codon and produces a molecule truncated in the α2-domain of exon 3 (14). The identification of healthy subjects homozygous for HLA-G null allele suggests that the HLA-G*0105N allele may generate other HLA-G isoforms such as membrane-bound HLA-G2 and -G3 and soluble HLA-G6 and -G7 proteins, which may substitute for HLA-G1 and -G5, thus assuming the immune tolerogeneic function of HLA-G. It is worth noting that this truncated allele can still perform certain functions such as providing a leader peptide for HLA-E expression (15).

Since HLA-G*0105N could maintain the presence of functional HLA-G proteins, the advantages of the selection of this allele must further be investigated in populations. Recent reports have described healthy individuals homozygous for this allele. The deletion of HLA-G1 membrane-bound protein at the fetal-maternal interface has been determined in a variety of ethnic groups with different frequencies of 7.4–8% in African-Americans, 4.8% in Ghanaians, 3% in Spaniards, 2.3% in mixed German-Croatians, 0.6% in Danish populations, and 11% in the Shona ethnic group of Zimbabwe (7,14,16-19). Furthermore, although the existence of HLA-G*0105N allele has been reported in different populations, no HLA-G*0105N allele has been found in Japanese or American Caucasian populations (7, 16). Additionally, the HLA-G*0105N allele is relatively frequent in African–Americans (7.4 %), while it has lower prevalence in Northern (0.6 %) and Middle Europeans (2.3 %) (1). In Amerindian population, from the Brazilian Amazon region no copy of this allele was found by exon 3 sequencing (20). Mendes-Junior supposed that the high frequency of HLA-G*0105N allele from the areas with high pathogen loads and the postulated role of HLA-G in placental development suggest that the reduced G1 expression in G*0105N heterozygous placentas may improve the intrauterine defense against infections (20). This study showed the existence of HLA-G*0105N allele in Iranian healthy subjects and that the frequency of the null allele was high. So far, different studies have demonstrated that the frequency of HLA-G*0105N null allele varies among different geographic regions. But the exact essence for this difference is unknown. Future research may reveal the importance of this allele in the populations and its possible role in normal or pathological conditions.

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