BRIEF COMMUNICATION
Iran J Allergy Asthma Immunol
June 2014; 13(3):207-213.

HLA-G allele and Haplotype Frequencies in a Healthy Population of Iran

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Received: 24 May 2013; Received in revised form: 25 July 2013; Accepted: 29 September 2013

ABSTRACT

The human leukocyte antigen (HLA)-G molecule is expressed in cytotrophoblast cells, adult thymic epithelial cells, erythroblasts, pancreatic islets and mesenchymal stem cells. Although, HLA-G expression in allotransplanted patients is correlated with a better allograft acceptance, it is associated with an advanced grade of the tumor in cancer. In addition to the role on the immune system, HLA-G is also involved in successful pregnancy through the embryo implantation, fetal survival and the initial steps of hematopoiesis and angiogenesis.

The aim of this study was determination of *HLA-G* allele frequencies in a healthy population of Iran. In this research, we selected 100 samples from healthy Iranian individuals and henceforth, we used polymerase chain reaction (PCR) followed by sequencing technique for exon 2, 3, 4 and intron 2 of the gene for evaluating the *HLA-G* alleles frequencies. Investigation of intronic (intron 2) variation is the novelty of our study.

The obtained results indicated thirteen alleles of *HLA-G* in Iranian individuals including G*01:01:01:01, G*01:06, G*01:01:01:06, G*01:01:02, G*01:01:03, G*01:01:05, G*01:01:06, G*01:01:07, G*01:01:08, G*01:03, G*01:04:01, G*01:04:03, and G*01:04:04. According to this study, the most prevalent alleles in the Iranian population were G*01:01:01:01 (52.5%), G*01:01:02 (16%) and G*01:04:03 (14.5%) and also the lowest alleles regarding the frequency were G*01:01:01:06 (0.5%) and G*01:03 (0.5%).

The results of G*01:01:01:01 and G*01:04:01 frequencies showed some similarities with the polish population. Our results were similar to the north Indian population for the frequencies of G*01:06 and G*01:01:02.

Keywords: Allele frequency; HLA-G; Iranian; Polymorphism; Population

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Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Human Leukocyte Antigen (*HLA*) complex contains over 150 loci that are located on chromosome 6p21. The product of these genes involves in regulating immune response and presenting peptides to T-cells, and is divided into three regions, class I, II and III. HLA class I includes: classical (Ia): HLA-A, HLA-B and HLA-C, non-classical (Ib): HLA-E, HLA-F and HLA-G and pseudogenes: HLA-H, HLA-J, HLA-K and HLA-L.[1] *HLA-G* gene exhibits 7 introns and 8 exons. Exons 2, 3 and 4 of the gene encode the extracellular domains (α1, α2 and α3) of protein. This gene presents 7 protein isoforms, 4 of them being membrane-bound (HLA-G1, G2, G3 and G4) and 3 soluble (G5, G6 and G7) isoforms that are generated by alternative splicing of the primary transcript.[2] HLA-G is expressed at the maternal-fetal interface by extravillous cytotrophoblast cells and endothelial cells of fetal vessels present in chorionic villi and it suppresses the maternal immune responses in this region.[3,4] Several recent studies have shown that there is a relationship between secretion of HLA-G in embryo culture and pregnancy outcome. One study reported detection of soluble HLA-G soluble HLA-G in embryo culture (EC) was significantly associated with pregnancy after assisted reproductive technique (ART).[5] Also, the expression of the molecule was shown in healthy tissues comprising cornea, adult thymic epithelial cells, erythroblasts, pancreatic islets, endothelial precursors and mesenchymal stem cells.[3,6] The gene has interactions with cell receptors of the immune system, including the killer cell immunoglobulin-like receptor KIR2DL4, CD8, LILRB1 and LILRB2.[7] To date, several functions were identified for this molecule including prevention of cytolytic killing, apoptosis induction and cytokine production in natural killer cells (NK). *HLA* polymorphism has been associated with several disorders, including preeclampsia, recurrent spontaneous abortion (RSA), autoimmune disease (lupus erythematosus, multiple sclerosis and rheumatoid arthritis patients)[8] and pemphigus vulgaris.[9] Viruses can impede NK cell recognition via down regulation of HLA-G expression in virus-infected cells.[8,10] Studies looking at the association between HLA-G expression and HIV-1 infection showed that the *HLA-G**01:01:08 allele was associated with susceptible to HIV-1 infection in Zimbabwean women, whereas in the *HLA-G**01:05N allele carriers had been observed significant protection against HIV-1 infection.[11,12] In other words, deficiency in modulation of NK cells Activity in individuals carrying the *HLA-G**01:05N allele, due to reduced expression of HLA-G molecules may be leading to protection against HIV-1 infection.[8] Recent studies have demonstrated that the increase in sHLA-G serum/plasma levels in the allograft were associated with better graft acceptance, increased graft survival or both.[13-15] Also, Crispim et al. reported that the 14-bp insertion (in exon 8) homozygous genotype had a relationship with acute transplant rejection.[16] Other study distributed lower risk of rejection in two HLA-G matches comparison to zero or one match in kidney transplantation.[17] According to studies, HLA-G expression has been detected in several tumor cells such as renal cell carcinomas,[18] ovarian carcinomas,[19] endometrial adenocarcinomas,[20] cutaneous T cell lymphomas[21] and pancreatic ductal adenocarcinoma.[22] So HLA-G has an important role in cancer immunoediting by inhibiting the cytotoxic functions of T and NK cells and decreasing the elimination of tumor cells.[8] To date, 50 alleles, 2 null alleles and 16 distinct proteins including (HLA-G*01:01, *01:02, *01:03, *01:04, *01:06, *01:07, *01:08, *01:09, *01:10, *01:11, *01:12, *01:14, *01:15, *01:16, *01:17 and *01:18) have been described to the *HLA-G* gene (http://hla.alleles.org/nomen clature/stats.html). The *HLA-G* alleles' frequencies have been studied in different populations exemplifying, north India, German, Polish and other populations. The goal of this research was to investigate the *HLA-G* gene polymorphisms in Iranian healthy population to be used as a documented reference for future studies on *HLA-G* variation and its association with unexplained infertility and other disorders.

MATERIALS AND METHODS

Study Subjects and DNA Extraction

We randomly collected 100 healthy individuals from Iranian native population. The genomic DNA was extracted and purified from peripheral blood cell samples using salting out procedure.[23] The concentration and purity of DNA was assessed by bio photometer.

HLA-G Allele Assignment

HLA-G genotyping was carried out using

polymerase chain reaction (PCR) followed by sequencing analysis. Briefly exon 2, 3 and intron 2 were amplified with a primer pair: forward 5′- GGC TGA GAG GTC TAC AGG AGA T-3′ and reverse 5′-GCT CCC ACT CCA TGA GGT ATT-3′and amplification of exon 4 was performed using the primers: forward 5′-GTA TCT GGT TCA TTC TTA GGA TGG-3′ and reverse 5′-AAG ACT GCT CTG GGA AAG G-3′. PCR product of exon 2, 3 and intron 2 was 822bp and also 502bp for exon 4. The polymerase chain reaction (PCR) program for exon 2, 3 and intron 2 was: after 95˚C for 10 min, 30 cycles of 94˚C for 1 min and 60˚C for 45 second followed by 72˚C for 45 second and for exon 4 was: after 95˚C for 5 min, 30 cycles of 94˚C for 1 min and 59˚ C for 45 second followed by 72˚C for 45 second. Moreover, the products were sequenced and 'plink' software was used (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) for allelic haplotyping.

Statistical Analysis

Statistical analysis was performed with the SPSS software (version 18.0; SPSS, Chicago, IL).

RESULTS

Based on nucleotide sequence variations in *HLA-G* exons 2 to 4, we identified thirteen different alleles in healthy individuals (Table 1). The order of frequencies of *HLA-G* alleles in this population was as follows: G*01:01:01:01 (52.5%), G*01:06 (2.5%), G*01:01:01:06 (0.5%), G*01:01:02 (16%), G*01:01:03 (0.5%), G*01:01:05 (2%), G*01:01:06 (0.5%) , G*01:01:07 (3%), G*01:01:08 (2.5%), G*01:03 (1.5%), G*01:04:01 (1.5%), G*01:04:03 (14.5%) and G*01:04:04 (2.5%). According to this study, the most prevalent alleles in the Iranian population were G*01:01:01:01 (105/200), G*01:01:02 (32/200) and G*01:04:03 (29/200) and also the alleles with the lowest frequency were G*01:01:01:06 (1/200) and G*01:03 (3/200). We observed 25-genotypes for *HLA-G* haplotypes. Most of the genotype frequencies belonged to G*01:01:01:01/*01:01:01:01 (n=37) and G*01: 04:03/*01:01:02 (n=17), and genotypes with the lowest frequencies belonged to G*01:03/ *01:01:02, G*01:06/*01:03, G*01:01:02/ *01:04:01, G*01:01:02/*01:01:08, G*01:04:04/ *01:01:07, G*01:04:04/*01:01:07, G*01:04:01/ *01:01:08, G*01:03/*01:01:01:01, G*01:04:04/ *01:01:02, G*01:04:03/*01:01:06, G*01:01:05/ *01:06, G*01:01:01:06/*01:04:01, G*01:04:03/ *01:04:03, G*01:04:03/*01:01:03 and G*01:01: 02/*01:06 (number of either genotype=1). Table 2 shows the *HLA-G* individuals' genotype frequencies (%) in the Iranian population.

Table 1. HLA-G allele frequencies in Iranian healthy population

HLA-G alleles	Healthy population, n (%)
G*01:01:01:01	105 (52.5)
G*01:06	5 (2.5)
G*01:01:01:06	1 (0.5)
G*01:01:02	32 (16)
G*01:01:03	1 (0.5)
G*01:01:05	4 (2)
G*01:01:06	1 (0.5)
G*01:01:07	6 (3)
G*01:01:08	5 (2.5)
G*01:03	3 (1.5)
G*01:04:01	3 (1.5)
G*01:04:03	29 (14.5)
G*01:04:04	5 (2.5)
All alleles	200 (100)

DISCUSSION

We analyzed *HLA-G* genotypes in 100 Iranian healthy individuals using sequencing follow by *HLA* typing method with high resolution and then its frequencies were compared with other populations.

In this study, we observed the presence of only thirteen of the 50 known *HLA-G* alleles. The data indicate that the Iranian healthy population has limited allelic variation. *HLA-G* allele frequencies in Iranians were more similar to those reported in Danish population compared to Polish and other populations (Table 3).[24-33] In our data, G*01:01:03 and G*01:04:01 showed the lowest frequencies compared with other populations, and G*01:01:01:01 showed higher frequency than those of most world populations. The G*01:01:03 allele has a synonymous substitution at the third base of codon 107 in exon 3 of the gene (Adenine to Thymine), that encodes glycine. The G*01:04:01 has substitution at the first base of codon 110 in exon 3 of the gene (cytosine to adenine) that led to change of leucine codon to isoleucine.[8] A study reported that HLA-G has an important role in pregnancy outcome.[4] For as much as this SNP is located on α_2 domain from HLA-G

Table 2. HLA-G genotype frequencies (%) in Iranian healthy population (individuals' haplotype, n = 100)

Genotype	Number	Genotype	Number
G*01:01:01:01/*01:01:01:01	37	G*01:01:02/*01:04:01	1
G*01:04:03/*01:01:02	17	G*01:01:02/*01:01:08	1
G*01:01:02/*01:01:01:01	8	G*01:04:04/*01:01:07	1
G*01:04:03/*01:01:01:01	6	G*01:04:01/*01:01:08	1
G*01:04:04/*01:01:01:01	3	G*01:03/*01:01:01:01	1
G*01:01:07/*01:01:01:01	3	G*01:04:04/*01:01:02	1
G*01:01:05/*01:01:01:01	3	G*01:04:03/*01:01:06	1
G*01:01:08/*01:01:01:01	3	G*01:01:05/*01:06	1
G*01:01:01:01/*01:04:03	2	G*01:01:01:06/*01:04:01	1
G*01:06/*01:01:01:01	2	G*01:04:03/*01:04:03	1
G*01:01:02/*01:01:07	2	G*01:04:03/*01:01:03	1
G*01:03/*01:01:02	1	G*01:01:02/*01:06	1
G*01:06/*01:03	1		

protein and this domain is involved in peptide presentation,[8] we suggested that probably lower frequency of the allele comparison to other population contributed to successful pregnancy, because the study individuals were selected among healthy individuals, however decisive justification is needed by more studies. Several studies reported that there are relationship between the 14-bp insertion / deletion in exon 8 of *HLA-G* and some disorders such as recurrent miscarriage and preeclampsia.[34,35] On the other side, it has been reported that G*01:01:08 has a linkage disequilibrium with 14-bp deletion at 3'UTR in Korean population.[36]

*HLA-G*01:04:03 allele (14.5%) seems to be a common allele in Iranian than other populations and this allele was not observed in Brazilian, Korean, Danish and Polish populations. Moreover, *HLA-*G*01:01:02 allele (16%) showed lower frequency in Iranian population compared to Finnish, German, Portuguese, Polish, Danish, Hutterite, North India and Brazilian populations, and also showed higher frequency in comparison with Korean, Chinese Han and Japanese populations (Table 3).

Table 3. HLA-G allele frequencies (%) in the present study compared with different studies

Allele	Iranian[a]	Spanish	Portuguese	Brazilian	Hutterite	Korean	Finnish	North India	Danish	Polish	Chinese Han	German	Japanese
	(n=100)	(n=228)	(n=117)	(n=103)	(n=80)	(n=200)	(n=194)	(n=120)	(n=198)	(n=100)	(n=292)	(n=82)	(n=344)
G*01:01:01:01	52.5	38.0	37.0	39.8	46.00	42.5	58.0	10.0	58.0	52.00	37.3	43.0	32.0
G*01:06	2.5	-	-	4.9	-	0.8	-	2.9	2.0	0	-	-	-
G*01:01:01:06	0.5	-	-	-	-	-	-	-	-	-	-	-	-
G*01:01:02	16.0	22.0	31.0	19.9	20.0	10.8	38.0	16.3	25.0	30.5	11.6	14.0	36.0
G*01:01:03	0.5	7.0	17.0	5.3	2.0	5.0	5.0	5.0	4.7	5.0	20.2	5.0	7.0
G*01:01:05	2.0	-	-	0	-	0	-	0	0	0	-	-	-
G*01:01:06	0.5	-	-	1.0	-	0	-	-	-	4.0	-	-	-
G*01:01:07	3.0	-	-	0	-	0	-	0	0	0	-	-	-
G*01:01:08	2.5	-	-	4.4	-	3.3	-	0	0.5	4.5	5.5	-	9.0
G*01:03	1.5	0	2.0	8.7	2.7	0.5	-	24.2	2.0	0	0.3	-	2.3
G*01:04:01	1.5	11.0	13.0	8.3	13.0	34.0	-	17.5	7.0	2.0	18.5	38.0	6.0
G*01:04:03	14.5	-	-	0	-	0	-	-	0	0	2.7	-	-
G*01:04:04	2.5	-	-	3.9	-	0	-	-	-	0	-	-	-
01:05N	0	3.0	0	1.0	-	2.3	-	15.4	0.8	1.5	1.4	0	2.3

a: This study –: Not determined

G*01:04:03 allele has the same SNP as G*01:04:01 but since its allele frequency was observed more than nine times to G*01:04:01, It is likely that G*01:04:03 has a linkage disequilibrium with polymorphisms in other regions such as 3'UTR or 5'UTR of the gene. Other data indicated that the frequency of G*01:03 (1.5%) was approximately similar to those reported in Danish and Portuguese (2%), German (2.3%), Hutterite (2.7%) rather than other populations (Table 3).

The previous study expressed an association between G*01:04 allele family and progression to high-grade bladder tumor and also between G*01:03 allele and protection against transitional cell carcinoma (TCC).[37] In the present study, frequencies of G*01:04 allele was 18.5% and for G*01:03 allele was 1.5%. Since bladder cancer (BC) is the third most common cancer in Iranian population and also the most common type of tumor was TCC (38), probably high frequency of G*01:04 allele in Iranians is the cause of this susceptibility to BC and TCC.

No *HLA-G**01:05N allele was detected in this group. *G**01:05N Allele is defined by a cytosine deletion at exon 3 that leading to change of reading frame and create a stop codon at exon 4 of the gene. Previous reports showed that the G*01:05N allele is associated with recurrent abortion.[4,8] The absence of this allele in this group could represent an important association between *G**01:05N and pregnancy failure and perhaps for this reason it was not detected in healthy group, though more researches are needed to fully establish this concept. Previous studies have shown that the frequency of *HLA-G* alleles have considerable variation in different racial and ethnic populations. In our data and the recent researches, *HLA-G* coding regions (exon 2, 3 and 4) showed low polymorphisms as well as intron 2 of the gene. Therefore, determined human *HLA-G* location has been conserved. Although the association between G* alleles with some disease was expressed but it is more reliable to explore the linkage disequilibrium between *HLA-G* and 3'UTR (exon 8) or 5'UTR of the gene and also other *HLA* alleles. Hence, the increase of sHLA-G serum/plasma levels were associated with graft success and pregnancy outcome, We suggest that the relationship between G* polymorphisms and its expression level should be investigated in the future.

ACKNOWLEDGEMENTS

The authors thanks to Genetic department staff of Royan Institute (Tehran, Iran) for the technical support.

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