Title
High-resolution HLA-A, HLA-B, and HLA-DRB1 haplotype frequencies from the French Bone Marrow Donor Registry

Permalink
https://escholarship.org/uc/item/93b5r920

Journal
Human Immunology, 76(5)

ISSN
0198-8859

Authors
Gourraud, PA
Pappas, DJ
Baouz, A
et al.

Publication Date
2015-05-01

DOI
10.1016/j.humimm.2015.01.028

Peer reviewed
Title: An investigation on association of HLA-G 14bp insertion/deletion polymorphism to multiple sclerosis susceptibility

Article Type: Research Article

Keywords: MS; HLA-G; insertion/deletion polymorphism; real time PCR, HRM

Corresponding Author: Mr. nabi mohammadi, msc

Corresponding Author's Institution: Faculty of Medicine Isfahan university of Medical Science

First Author: nabi mohammadi, msc

Order of Authors: nabi mohammadi, msc; Fereshteh Alsahebfosoul; Mohammad Kazemi; mino adib, phD; Fereshteh Alsahebfosoul, ph.D; Mohammad Kazemi, Msc

Abstract: Human Leukocyte Antigen G (HLA-G) gene polymorphism and expression rate have recently been suggested to have a potential role in susceptibility to Multiple Sclerosis (MS), a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system with unknown etiology. The aim of this study was to investigate the association of the frequency of HLA-G gene 14bp insertion/deletion polymorphism and its plasma level to MS susceptibility. In this study, HLA-G gene from 212 patients and 210 healthy individuals was amplified using real time PCR and screened for the 14bp insertion/deletion polymorphism. In addition, HLA-G plasma level of the patients were measured and compared to normal controls by ELISA method. Our results revealed that 14bp insertion in HLA-G could result in lower plasma HLA-G level of the subjects, regardless their health status and vice versa. Additionally, significant correlation of HLA-G genotype and its plasma level to MS susceptibility was observed. In conclusion, not only HLA-G 14bp insertion/deletion polymorphism could be associated to expression rate of the HLA-G gene and its plasma level, but also could be considered as a risk factor for susceptibility to MS in our study population.

Suggested Reviewers: Roberta Rizzo
Department of Experimental and Diagnostic Medicine, Section of Microbiology, University of Ferrara, Ferrara 44121, Italy
rbr@unife.it

Carole Ober
Department of Human Genetics, The University of Chicago, Chicago, IL, USA
c-ober@genetics.uchicago.edu

Eduardo A Donadi
Division of Clinical Immunology, Department of Medicine, eadonadi@fmrp.usp.br
An investigation on association of HLA-G 14bp insertion/deletion polymorphism to multiple sclerosis susceptibility

Nabiallah mohammadi MSc, Mino adib PhD, Fereshteh Alsahebfosoul PhD, Mohammad Kazemi MSc, Masoud Etemadifar

1 Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences

2 Department of Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences

3 Neurology Department, Faculty of Medicine, Isfahan University of Medical Sciences.

Corresponding author's e. mail address: Mino Adib PhD Department of Immunology, Faculty of Medical Sciences Isfahan University of Medical Sciences, Email: Minoo_adib@yahoo.com

Tel:+983137922497
An investigation on association of HLA-G 14bp insertion/deletion polymorphism to multiple sclerosis susceptibility

Nabiallah mohammadi MSc¹, Mino adib PhD¹ Fereshteh Alsahfosoul PhD¹ Mohammad Kazemi MSc², Masoud Etemadifar³

¹Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences

²Department of Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences

³Neurology Department, Faculty of Medicine, Isfahan University of Medical Sciences.

Corresponding author's e. mail address: Mino Adib PhD Department of Immunology, Faculty of Medical Sciences Isfahan University of Medical Sciences, Email: Minoo_adib@yahoo.com

Tel:+983137922497
Abstract

Human Leukocyte Antigen G (HLA-G) gene polymorphism and expression rate have recently been suggested to have a potential role in susceptibility to Multiple Sclerosis (MS), a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system with unknown etiology. The aim of this study was to investigate the association of the frequency of HLA-G gene 14bp insertion/deletion polymorphism and its plasma level to MS susceptibility. In this study, HLA-G gene from 212 patients and 210 healthy individuals was amplified using real time PCR and screened for the 14bp insertion/deletion polymorphism. In addition, HLA-G plasma level of the patients were measured and compared to normal controls by ELISA method. Our results revealed that 14bp insertion in HLA-G could result in lower plasma HLA-G level of the subjects, regardless their health status and vice versa. Additionally, significant correlation of HLA-G genotype and its plasma level to MS susceptibility was observed. In conclusion, not only HLA-G 14bp insertion/deletion polymorphism could be associated to expression rate of the HLA-G gene and its plasma level, but also could be considered as a risk factor for susceptibility to MS in our study population.

Keywords:
MS; HLA-G; insertion/deletion polymorphism; real time PCR.
**Introduction**

Multiple Sclerosis (MS) is a chronic inflammatory disease, affecting the central nervous system (CNS) and causing progressive and relapsing neurological disability, which involves demyelination and hard axonal damage (1). MS is currently hypothesized to be mediated by T-cell responses to myelin antigens (2). More than 2.3 million people are affected by the disease (National Multiple Sclerosis Society), commonly occurring among young adults between 20 to 40 years old (1).

The etiology of MS remains elusive but has been suggested to be affected by genetic and environmental factors. A large number of studies have reported the effect of genetic variation on susceptibility to MS (3, 4). The genetic factors have role in MS (4-6). Among the potential genetic host factors, evaluation of the association between HLA-G (Human Leukocyte Antigen-G) and MS have gained its interests (6, 7). HLA-G is induced in the course of inflammatory pathologies such as myositis (8), psoriatic skin lesions (9) as well as MS (10) and seems to play important functions at immunologically privileged sites such as the thymus (11) and the cornea (12).

HLA-G are non-classical class Ib HLA molecules with differences from other classical class I HLA (-A, -B and -C) molecules including: (a) limited protein diversity, (b) Membrane and secretory several isoforms that are produced by alternative splicing of the primary transcript, (c) unique molecular structure, presenting a reduced cytoplasmic tail, (d) modulation of the immune response, and (e) limited tissue expression (13). HLA-G exists as seven isoforms...
including four membrane-bound (HLA-G₁, -G₂, -G₃ and -G₄) as well as three secreted soluble (HLA-G₅, G₆ and -G₇) proteins.(13)

Genetic Polymorphisms in coding and non-coding regions of the HLA-G gene may potentially affect biological features of the molecule (13). HLA-G expression rate and plasma level is affected by polymorphisms in the promoter region as well as 3’ untranslated region, which modify interaction between the target gene and transcriptional or post transcriptional factors, respectively (13). Nucleotide variability in the coding region of the HLA-G gene may produce conformational changes in the molecule which influences its major functions including interaction with cell receptors, isoform production, modulation of immune response, polymerization features as well as ability to couple peptides (13). Recently, contribution of 14bp insertion/deletion polymorphism (rs16375) in exon 8 of the HLA-G gene to the risk and severity of MS has been evaluated. Effect of polymorphisms of the 5’ upstream regulatory and 3’ untranslated regions of the HLA-G gene to expression of HLA-G has been described(14). In addition, it was reported that the HLA-G 14bp insertion polymorphism in exon 8 increases mRNA stability (15) and is associated with pregnancy pathologies and autoimmune diseases (16, 17).

The aim of the present work was to evaluate prevalence of HLA-G 14bp insertion/deletion polymorphisms in a group of relapsing-remitting (RR)-MS patients compared with healthy individuals in order to clarify the impact of these genetic variations on HLA-G expression level in MS patients and susceptibility to MS.

**Materials and Methods**

Patients and controls
Two hundred and twelve patients (38 males and 174 females) affected with definite relapsing–remitting MS (RRMS) (mean age= 31.28±8.6 years) according to the classification of McDonald (2001) referring to MS clinic of Isfahan- Isfahan is in central Iran and the high prevalence of MS in the city- were involved in this study. Assessment of disease disability was performed in all MS patients using Kurtzke's Expanded Disability Status Scale (EDSS) and brain Magnetic resonance imaging(MRI). All RRMS patients were in clinical remission at the time of blood collection. At the time of inclusion in the study, 71 patients were not receiving immunomodulatory drugs, whereas the other patients were under treatment with interferon-β1a (IFN-β). Two hundred and ten healthy individuals (65 males and 145 females) of donors Isfahan Blood Transfusion Organization with the mean age of 32.2±7.48 were also involved in this study. Table 1 describes baseline characteristics of the patients and normal controls.

**Measurement Soluble HLA-G by Enzyme-Linked Immunosorbent Assay (ELISA)**

Samples comprising 5 ml of peripheral blood were collected from the patients and normal controls and centrifuged at 2400×g for 10 min. The resulting plasma were frozen immediately at -20°C and used for further studies. Plasma concentrations of Soluble HLA-G (sHLA-G) were quantified using a commercial Elisa kit (Hangzhou Eastbiopharm, Boster Biological Technology, china) according to the manufacturer's instruction. The optical density of samples was measured at 450 nm using Dynatech plate reader and sHLA-G levels were estimated following construction of calibration curve.

**Extraction of genomic DNA and Polymerase Chain Reaction (PCR) assay**

Genomic DNA samples were extracted from leukocytes in whole blood using (Amersham Pharmacia Biotech, Buckinghamshire, UK). DNA extraction kit according to the manufacturer's protocols. DNA concentration and purity was evaluated using UV
spectrophotometry and electrophoresis, respectively. HLA-G exon 8 was amplified using real-time PCR assay with forward and reverse primers of 5′-AGCATGTGATGGGCTTTT 3′ and 5′- AAGGTGATTGGGAAGGAAT3′, respectively. The assay was performed using the Rotor Gene 6000- Real-Time PCR System (Corebett research) and Feldan Real –Time PCR kit (Bio Basic, canada). Geotyping for 14bp insertion/deletion polymorphisms was performed (sequencing was performed by Biotech company for the initial and final approval was used as a reference) and 14bp insertion/deletion polymorphisms of the exon 8 was evaluated using High Resolution Melt (HRM) software (1.7 version).

**Statistical Analysis**

Statistical analysis was performed with the SPSS 13.0 software (SPSS, Inc., Chicago, IL). The normality of the variables was checked using the Kolmogorov–Smirnov test. Since, normality of data distribution was rejected in variables (P-Value<0.05), statistical analysis was performed using non-parametric analysis. Accordingly, Mann-Whitney U-test was used to compare mean sHLA-G plasma level between groups. In addition, sHLA-G concentrations in the case-matched samples compared to the respective 14bp insertion/deletion genotypes. P-values were determined, and those less than 0.05 were considered to be significant.

**Results**

sHLA-G Plasma concentration

sHLA-G plasma concentration of 212 RRMS patients and 210 healthy individuals were determined by ELISA. According to the results, sHLA-G levels in samples collected from RRMS patients (mean, 6.87±0.92 U/ml) were significantly higher than normal controls (mean, 4.59±1.14 U/ml)( p= 0.029, Fig.1). Moreover, it was found that there is inverse correlation
between sHLA-G plasma concentration and age in patients (P=0.049) while no correlation between sHLA-G plasma concentration and gender of the patients as well as controls (P=0.536, P=0.470) (Table 2).

**HLA-G 14bp insertion/deletion (INS/DEL) polymorphisms**

The frequency of the HLA-G alleles observed in the MS patients and controls was in Hardy-Weinberg equilibrium. Among 212 RRMS patients, prevalence of the genotype +14bp/+14bp, +14bp/-14bp and -14bp/-14bp were 30.5%, 30.5% and 39% and among 210 healthy individuals were 24.7%, 36.1% and 39.2%, respectively. Statistical analysis revealed no significant difference between patients and normal controls (P=0.556).

The alleles having 14bp insertion had the least prevalence (46% and 42.8% frequency for RRMS patients and controls, respectively), whereas 14bp deletion alleles were the most frequent alleles with 54% and 57.2% frequency in patients and controls, respectively (Table 3).

**Correlation of sHLA-G levels with 14bp insertion/deletion polymorphism**

Correlation of sHLA-G levels with its case-matched 14bp insertion/deletion genotypes was evaluated. The mean values of sHLA-G concentration in the RRMS patients were 5.10±1.30 U/ml, 5.90±1.37, and 10.47±2.61 U/ml for the genotypes of +14bp/+14bp, +14bp/-14bp and -14bp/-14bp, respectively. sHLA-G levels in the plasma from healthy individuals were 2.14±0.41, 4.35±2.27 and 10.50±3.48 for the mentioned genotypes, respectively (Table 3).

**Discussion**
According to our data, an association between HLA-G genotype and its plasma level was observed. Individuals with -14bp/-14bp had significantly higher level of sHLA-G plasma molecules in comparison to +14/+14 genotype, regardless their health status (P<0.05), showing a negative correlation between 14bp insertion of HLA-G gene and its expression level. In addition, sHLA-G plasma levels of patients were significantly higher than normal controls (P=0.029), which confirm a possible link between sHLA-G expression level and susceptibility to MS.

A large number of studies indicated that the HLA-G polymorphisms might be associated with several diseases. Several studies have focused on the contribution of genetic factors to MS susceptibility. Although MS is associated with polygenic involvement, an association between the HLA system and MS has been indicated (18, 19). The association of HLA-G genotype to MS susceptibility has been studied by several researchers but the results were controversial. Some studies associated 14bp insertion/deletion HLA-G gene polymorphisms and susceptibility to MS. Cree et al (5) confirmed the contribution of the MHC locus to MS susceptibility, not only through the well recognized effect of HLA-DRB1*15:01, but also through the rs4959039 single nucleotide polymorphism (SNP) in the 3’ untranslated region (UTR) of the HLA-G gene. Likewise, an association of 14bp insertion/deletion polymorphism to MS susceptibility was described by Wisniewski et al (21). Conversely, Kroner et al (19) did not identify any association of HLA-G gene polymorphisms to MS susceptibility and severity of disease. Similar results were also reported by Rizzo et al (22).

Thus, in the current work the association of HLA-G genotype and its expression rate to MS susceptibility was evaluated. HLA-G exon 8 was genotyped in order to evaluate presence of a 14bp insertion/deletion polymorphism and its association to HLA-G plasma level and susceptibility to MS.
In addition, it was found that there is inverse correlation between sHLA-G plasma concentration and age in patients (P=0.049) while no correlation between sHLA-G plasma concentration and gender of the patients as well as controls. (P=0.536, P=0.470)

According to our data, a 14bp deletion polymorphism of HLA-G exon 8, is implicated in higher expression rate of HLA-G gene and causing an increase of sHLA-G plasma level. In addition, an association of sHLA-G plasma concentration to MS susceptibility was observed, thus it could be suggested that there is a possible link between 14bp deletion polymorphism of HLA-G gene and an increased susceptibility to the disease. This finding is in accordance to the results reported by Cree et al(5) and Wisniewski et al(21).

**Conclusion**

It could be concluded that, HLA-G 14bp insertion/deletion polymorphism plays an important role in expression rate of the gene as well as sHLA-G plasma level. As a result, it could be suggested that, this polymorphisms may be associated with susceptibility to MS In our studied population. However, more investigations are needed to evaluate HLA-G polymorphisms in details and transcription and translation rate of HLA-G gene under different pathological and normal control conditions.

**Acknowledgments**

The authors would like to thank the Isfahan MS and Phisology Researches Center. We are grateful to all patients and volunteers who participated in this study and Isfahan University of Medical Sciences for providing facilities to carry out this work.

**References**

Fig 1. sHLA-G level in RRMS and Healthy controls.

P.Value = 0.029
Table 1. Baseline characteristics in two groups

<table>
<thead>
<tr>
<th>Factors</th>
<th>RRMS(n=212)</th>
<th>Healthy controls(n=210)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.28±8.63</td>
<td>32.22±7.70</td>
<td>0.242</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 38(17.9%)</td>
<td>65(31%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female 174(82.1%)</td>
<td>145(69%)</td>
<td></td>
</tr>
</tbody>
</table>

* Data showed by n(%) or Mean±SD and tests used t-test, Fisher exact and chi square($\chi^2$)

Table 2. Factors effect on sHLA-G in RRMS group & healthy group

<table>
<thead>
<tr>
<th>Variables</th>
<th>RRMS group</th>
<th>healthy group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sHLA-G</td>
<td>P-Value</td>
</tr>
<tr>
<td>Age (correlation)</td>
<td>-0.266</td>
<td>0.049</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 8.71±2.44</td>
<td>0.536</td>
</tr>
<tr>
<td></td>
<td>Female 6.42±0.98</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>14bp INS/INS 5.10±1.30</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>14bp INS/DEL 5.90±1.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14bp DEL/DEL 10.47±2.61</td>
<td></td>
</tr>
</tbody>
</table>

* Data showed Mean±SEM and tests used used Spearman Correlation, Mann-Whitney, Kruskal-Walis
Table 3. Frequencies of the 14 bp insertion/deletion polymorphism of HLA-G gene and sHLA-G level in patients and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>RRMS (n=212)</th>
<th>Healthy controls (n=210)</th>
<th>OR (CI95%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sHLA-G</td>
<td>6.87±0.92</td>
<td>4.59±1.14</td>
<td>-</td>
<td>0.036</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14bpINS/INS</td>
<td>65(30.5%)</td>
<td>52(24.7%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>14bpINS/DEL</td>
<td>65(30.5%)</td>
<td>76(36.2%)</td>
<td>1.45(0.73-2.88)</td>
<td>0.556</td>
</tr>
<tr>
<td>14bpDEL/DEL</td>
<td>82(39%)</td>
<td>82(39.1%)</td>
<td>1.22(0.84-1.79)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14bp INS</td>
<td>130(46%)</td>
<td>128(42.8%)</td>
<td>1.23(0.63-2.39)</td>
<td>0.036</td>
</tr>
<tr>
<td>14bp DEL</td>
<td>147(54%)</td>
<td>111(57.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data showed by n(%) or Mean±SEM and tests used Mann-Whitney and chi square ($\chi^2$)
Fig 1. sHLA-G level in RRMS and Healthy controls

P. Value = 0.029
Table 1. Baseline characteristics in two groups

<table>
<thead>
<tr>
<th>Factors</th>
<th>RRMS(n=212)</th>
<th>Healthy controls(n=210)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.28±8.63</td>
<td>32.22±7.70</td>
<td>0.242</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 38(17.9%)</td>
<td>65(31%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female 174(82.1%)</td>
<td>145(69%)</td>
<td></td>
</tr>
</tbody>
</table>

* Data showed by n(%) or Mean±SD and tests used t-test, Fisher exact and chi square($\chi^2$)

Table 2. Factors effect on sHLA-G in RRMS group & healthy group

<table>
<thead>
<tr>
<th>Variables</th>
<th>RRMS group</th>
<th>Healthy group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(correlation)</td>
<td>-0.266</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.049</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8.71±2.44</td>
<td>4.20±1.61</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.536</td>
</tr>
<tr>
<td>Female</td>
<td>6.42±0.98</td>
<td>4.95±1.67</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14bp INS/INS</td>
<td>5.10±1.30</td>
<td>2.14±0.41</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.088</td>
</tr>
<tr>
<td>14bp INS/DEL</td>
<td>5.90±1.37</td>
<td>4.35±2.27</td>
</tr>
<tr>
<td>14bp DEL/DEL</td>
<td>10.47±2.61</td>
<td>10.50±3.48</td>
</tr>
</tbody>
</table>

* Data showed Mean±SEM and tests used Spearman Correlation, Mann-Whitney, Kruskal-Walis
Table 3. Frequencies of the 14 bp insertion/deletion polymorphism of HLA-G gene and sHLA-G level in patients and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>RRMS (n=212)</th>
<th>Healthy controls (n=210)</th>
<th>OR (CI 95%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sHLA-G</td>
<td>6.87±0.92</td>
<td>4.59±1.14</td>
<td>-</td>
<td>0.036</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14bpINS/INS</td>
<td>65 (30.5%)</td>
<td>52 (24.7%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>14bpINS/DEL</td>
<td>65 (30.5%)</td>
<td>76 (36.2%)</td>
<td>1.45 (0.73-2.88)</td>
<td>0.556</td>
</tr>
<tr>
<td>14bpDEL/DEL</td>
<td>82 (39%)</td>
<td>82 (39.1%)</td>
<td>1.22 (0.84-1.79)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14bp INS</td>
<td>130 (46%)</td>
<td>128 (42.8%)</td>
<td>1.23 (0.63-2.39)</td>
<td>0.036</td>
</tr>
<tr>
<td>14bp DEL</td>
<td>147 (54%)</td>
<td>111 (57.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data showed by n(%) or Mean±SEM and tests used Mann-Whitney and chi square (χ²)