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

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Cover Letter

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

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

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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-G_1, -G_2, -G_3 and -G_4) as well as three secreted soluble (HLA-G_5, G_6 and -G_7) 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′-AGCATGTGATGGGCTGTTTA- 3′ and 5′- AAGGTGATTGGGGAAGGAAT3′, 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). Geontyping 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) soft ware (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±0.92U/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, and10.47±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 and10.50±3.48 for the mentioned genotypes, respectively (Table3).

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.

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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.

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References

1. Allen IV. The pathology of multiple sclerosis--fact, fiction and hypothesis. Neuropathology and applied neurobiology. 1981 May-Jun;7(3):169-82. PubMed PMID: 7242846. Epub 1981/05/01. eng.

2. Weiner HL. Multiple sclerosis is an inflammatory T-cell-mediated autoimmune disease. Arch Neurol. 2004 Oct;61(10):1613-5. PubMed PMID: 15477521. Epub 2004/10/13. eng.
3. Oksenberg JR, Baranzini SE, Barcellos LF, Hauser SL. Multiple sclerosis: genomic rewards. Journal of neuroimmunology. 2001 Feb 15;113(2):171-84. PubMed PMID: 11164900. Epub 2001/02/13. eng.
4. Ebers GC, Sadovnick AD, Risch NJ. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. Nature. 1995 Sep 14;377(6545):150-1. PubMed PMID: 7675080. Epub 1995/09/14. eng.
5. Cree BA, Rioux JD, McCauley JL, Gourraud PA, Goyette P, McElroy J, et al. A major histocompatibility Class I locus contributes to multiple sclerosis susceptibility independently from HLA-DRB1*15:01. PloS one. 2010;5(6):e11296. PubMed PMID: 20593013. Pubmed Central PMCID: PMC2892470. Epub 2010/07/02. eng.
6. Munoz-Culla M, Irizar H, Otaegui D. The genetics of multiple sclerosis: review of current and emerging candidates. The application of clinical genetics. 2013;6:63-73. PubMed PMID: 24019748. Pubmed Central PMCID: PMC3760455. Epub 2013/09/11. eng.
7. Catamo E, Addobbati C, Segat L, Sotero Fragoso T, Domingues Barbosa A, Tavares Dantas A, et al. HLA-G gene polymorphisms associated with susceptibility to rheumatoid arthritis disease and its severity in Brazilian patients. Tissue antigens. 2014 Jun 23. PubMed PMID: 24957665. Epub 2014/06/25. Eng.
8. Wiendl H, Behrens L, Maier S, Johnson MA, Weiss EH, Hohlfeld R. Muscle fibers in inflammatory myopathies and cultured myoblasts express the nonclassical major histocompatibility antigen HLA-G. Annals of neurology. 2000 Oct;48(4):679-84. PubMed PMID: 11026456. Epub 2000/10/12. eng.
9. Aractingi S, Briand N, Le Danff C, Viguier M, Bachelez H, Michel L, et al. HLA-G and NK receptor are expressed in psoriatic skin: a possible pathway for regulating infiltrating T cells? The American journal of pathology. 2001 Jul;159(1):71-7. PubMed PMID: 11438456. Pubmed Central PMCID: PMC1850403. Epub 2001/07/05. eng.
10. Wiendl H, Feger U, Mittelbronn M, Jack C, Schreiner B, Stadelmann C, et al. Expression of the immune-tolerogenic major histocompatibility molecule HLA-G in multiple sclerosis: implications for CNS immunity. Brain : a journal of neurology. 2005 Nov;128(Pt 11):2689-704. PubMed PMID: 16123145. Epub 2005/08/27. eng.
11. Crisa L, McMaster MT, Ishii JK, Fisher SJ, Salomon DR. Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. The Journal of experimental medicine. 1997 Jul 21;186(2):289-98. PubMed PMID: 9221758. Pubmed Central PMCID: PMC2198976. Epub 1997/07/21. eng.
12. Le Discorde M, Moreau P, Sabatier P, Legeais JM, Carosella ED. Expression of HLA-G in human cornea, an immune-privileged tissue. Human immunology. 2003 Nov;64(11):1039-44. PubMed PMID: 14602233. Epub 2003/11/07. eng.
13. Donadi EA, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. Cellular and molecular life sciences : CMLS. 2011 Feb;68(3):369-95. PubMed PMID: 21107637. Pubmed Central PMCID: PMC3021195. Epub 2010/11/26. eng.
14. Hviid TV, Rizzo R, Melchiorri L, Stignani M, Baricordi OR. Polymorphism in the 5' upstream regulatory and 3' untranslated regions of the HLA-G gene in relation to soluble HLA-G and IL-10 expression. Human immunology. 2006 Jan-Feb;67(1-2):53-62. PubMed PMID: 16698426. Epub 2006/05/16. eng.
15. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. American journal of human genetics. 2007 Oct;81(4):829-34. PubMed PMID: 17847008. Pubmed Central PMCID: PMC2227932. Epub 2007/09/12. eng.

16. Yan WH, Lin A, Chen XJ, Dai MZ, Gan LH, Zhou MY, et al. Association of the maternal 14-bp insertion polymorphism in the HLA-G gene in women with recurrent spontaneous abortions. Tissue antigens. 2006 Dec;68(6):521-3. PubMed PMID: 17176444. Epub 2006/12/21. eng.
17. Hviid TV, Christiansen OB. Linkage disequilibrium between human leukocyte antigen (HLA) class II and HLA-G--possible implications for human reproduction and autoimmune disease. Human immunology. 2005 Jun;66(6):688-99. PubMed PMID: 15993714. Epub 2005/07/05. eng.
18. Hensiek AE, Sawcer SJ, Feakes R, Deans J, Mander A, Akesson E, et al. HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 2002 Feb;72(2):184-7. PubMed PMID: 11796767. Pubmed Central PMCID: PMC1737743. Epub 2002/01/18. eng.
19. Kroner A, Grimm A, Johannssen K, Maurer M, Wiendl H. The genetic influence of the nonclassical MHC molecule HLA-G on multiple sclerosis. Human immunology. 2007 May;68(5):422-5. PubMed PMID: 17462509. Epub 2007/04/28. eng.
20. Link J, Kockum I, Lorentzen AR, Lie BA, Celius EG, Westerlind H, et al. Importance of human leukocyte antigen (HLA) class I and II alleles on the risk of multiple sclerosis. PloS one. 2012;7(5):e36779. PubMed PMID: 22586495. Pubmed Central PMCID: PMC3346735. Epub 2012/05/16. eng.
21. Wisniewski A, Bilinska M, Klimczak A, Wagner M, Majorczyk E, Nowak I, et al. Association of the HLA-G gene polymorphism with multiple sclerosis in a Polish population. International journal of immunogenetics. 2010 Aug;37(4):307-11. PubMed PMID: 20636826. Epub 2010/07/20. eng.
22. Rizzo R, Bortolotti D, Fredj NB, Rotola A, Cura F, Castellazzi M, et al. Role of HLA-G 14bp deletion/insertion and +3142C>G polymorphisMS in the production of sHLA-G molecules in relapsing-remitting multiple sclerosis. Human immunology. 2012 Nov;73(11):1140-6. PubMed PMID: 22922127. Epub 2012/08/28. eng.

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

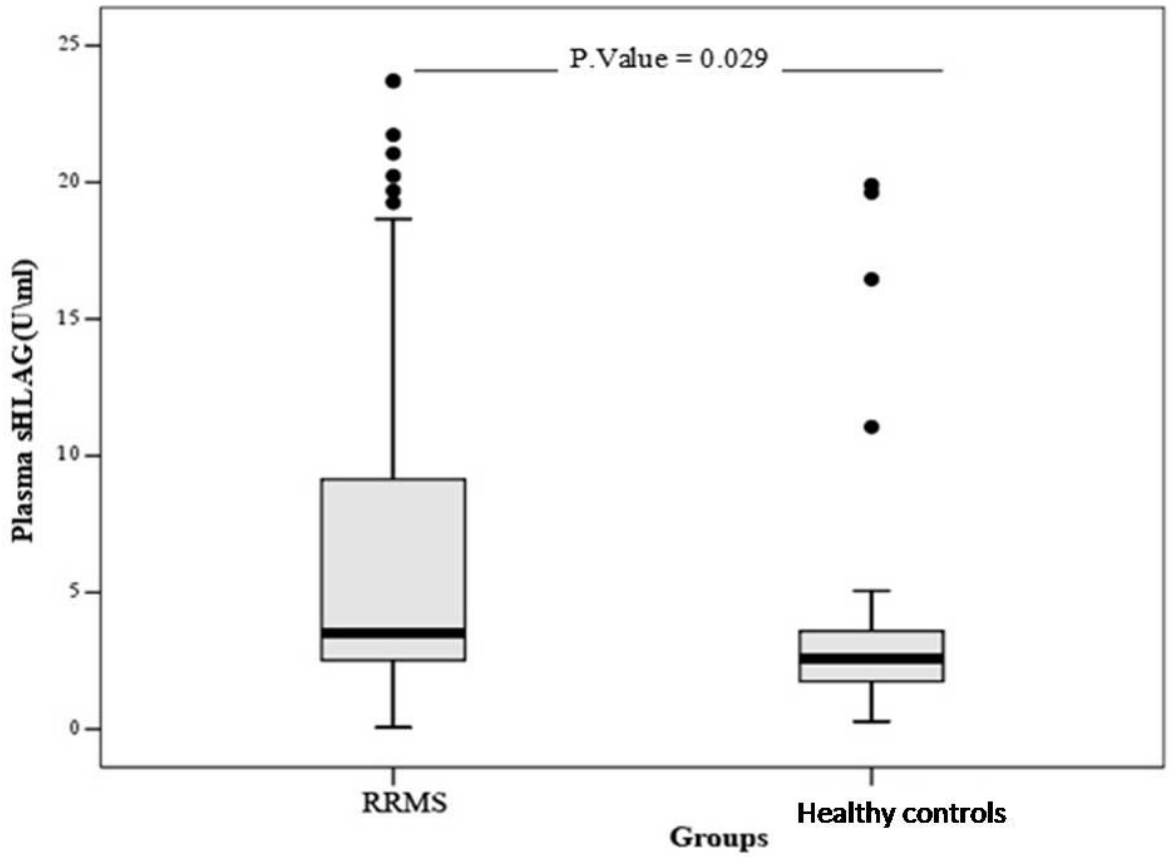

Table1. Baseline characteristics in two groups

Factors		RRMS(n=212)	Healthy controls(n=210)	P-Value
Age		31.28±8.63	32.22±7.70	0.242
Sex	**Male**	38(17.9%)	65(31%)	
	Female	174(82.1%)	145(69%)	

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

Table2. Factors effect on sHLA-G in RRMS group & healthy group

	RRMS group		healthy group		
Variables	**sHLA-G**	**P-Value**	**sHLA -G**	**P-Value**	
Age(correlation)	-0.266	0.049	0.020	0.925	
Sex					
Male	8.71±2.44	0.536	4.20±1.61	0.470	
Female	6.42±0.98		4.95±1.67		
Genotype					
14bp INS/INS	5.10±1.30	0.088	2.14±0.41	0.036	
14bp INS/DEL	5.90±1.37		4.35±2.27		
14bp DEL/DEL	10.47±2.61		10.50±3.48		

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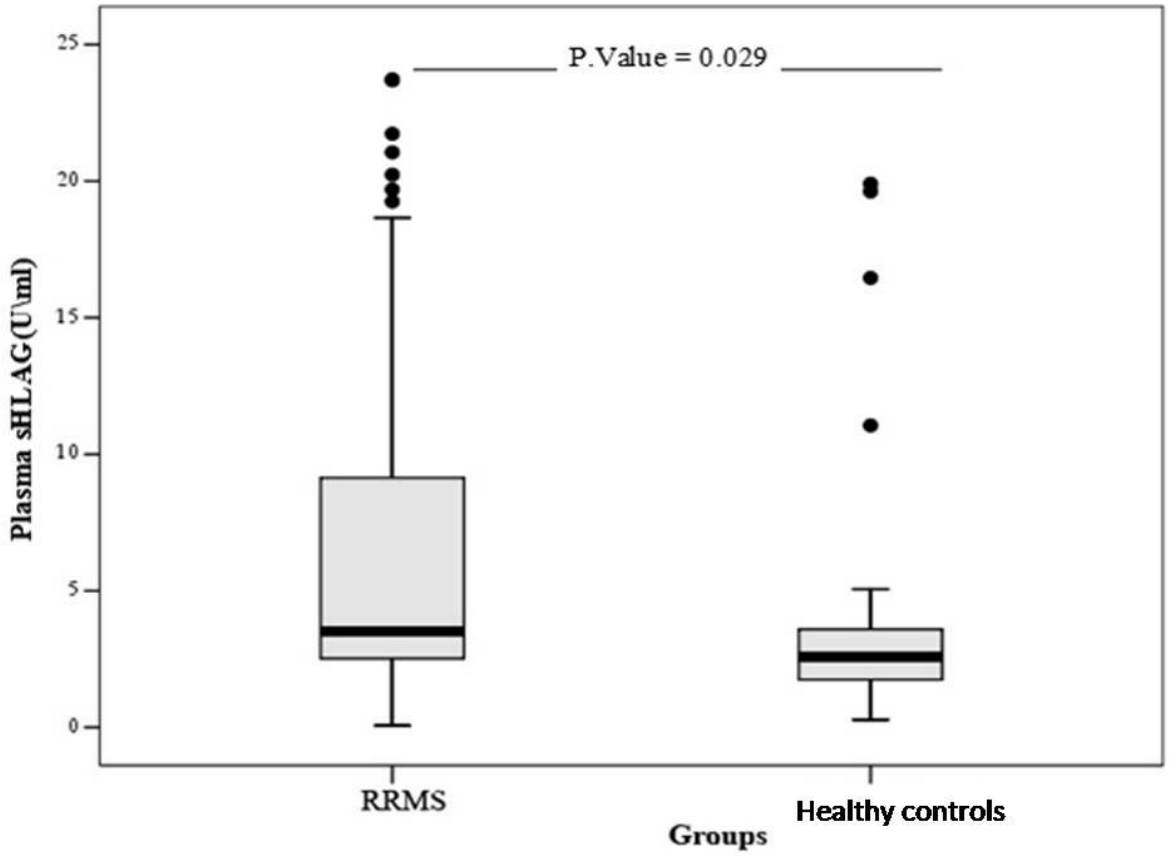
Table3. Frequencies of the 14 bp insertion/deletion polymorphism of HLA-G gene and sHLA-G level in patients and healhy controls

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

* Data showed by n(%) or Mean±SEM and tests used Mann-Whitney and chi square(χ^2)

Figure(s)

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



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