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HLA-G 14bp Deletion/Insertion Polymorphisms in Multiple Sclerosis

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Abstract

Background

The intensive researches during the last 40 years have found the human major histocompatibility complex (HLA) as the only locus conclusively associated with multiple sclerosis (MS). Recently, a possible influence of HLA-G in MS has been suggested due to its significant role in immune tolerance. One of them, HLA-G 14bp INS/DEL, has not been intensively studies and the published studies reported controversial results.

Aim

The aim of the present study was to examine the association of HLA-G 14bp INS/DEL and MS.

Methods

We present a case control study with 51 patients (familial MS 39%) and 51 healthy controls. All cases were with definite MS according to McDonald's criteria. The analysis of HLA-G 14bp INS/DEL was performed by PCR of DNA from peripheral blood.

Results

Overall comparison did not reveal statistically significant association between HLA-G 14bp INS/DEL and MS. A higher frequency of 14bpINS allele (60% vs 43%) and INS/INS genotype (40% vs 24%) was noted in the familial versus sporadic cases and controls. A significant correlation was found for genotype INS/INS in females. Surprising and reciprocal to the literature results were found in males. Genotype INS/DEL (high producers) was associated with higher risk, whereas INS/INS (low producers) was found to be protective.

Conclusion

HLA-G 14bp INS/INS is likely to be involved in familial MS and females in our population. The finding in males should be interpreted with a caution. The results warrant additional studies with international collaboration and larger sample size.

Keywords: multiple sclerosis, HLA-G 14bp INS/DEL.

Introduction

Multiple sclerosis (MS) is thought to be a result of complex interactions between unknown genetic and environmental factors. The intensive researches during the last 40 years have found the human major histocompatibility complex (HLA) as the only locus conclusively associated with MS (1). Although HLA I alleles - A*03 and B*07 were firstly reported, the strongest association has been found only with HLA-DRB1*15:01[1,2].

During the last decade, a possible role of HLA-G has been suggested due to its role in the regulation of the immune tolerance.[3-5] The role of different polymorphisms such as HLA-G 14bp INS/DEL has not been intensively studied and the preliminary studies reported controversial results. Therefore we sought to examine the role HLA-G 14bp INS/DEL and MS in a small pilot case-control study.

Material and methods

A case control study with 51 patients with definite MS diagnose by Mc Donald's criteria and 51 sex and age matched healthy controls was conducted. The disease course, long term disability and family history were assessed at the time of the sample collection. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from all subjects prior to genetic testing.

DNA testing

DNA was extracted from peripheral blood. PCR primers was used to genotyped HLA-G 14 bp INS/DEL polymorphism (rs66554220).[6] Genomic DNA (100 ng) were amplified in a 25 μl reaction with a final concentration of reaction buffer (Genet Bio, Korea) 1×; each deoxynucleoside triphosphate 0.2 mmol/L; Taq polymerase (Genet Bio, Korea) 0.5 U and 0.5 μM for each primer. PCR amplification was performed with initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 45 s, 61 °C for 45 s and 72 °C for 1 min and subsequently at 72 °C for 10 min. The products were visualized by electrophoresis on a 3% agarose gel (Applichem, Germany) containing ethidium bromide (0.5 μg/mL). PCR products were either 224 or 210bp, or both 224 and 210bp, according to the insertion/deletion of the 14bp in exon 8. The observed genotypes were directly counted by two different observers.

Statistics

Family history, clinical course and HLA-G 14 bp polymorphism were analyzed. We compared HLA-G 14bp allelic and genotype distribution between patients and healthy subjects, between sporadic and family cases and in subsets of patients with different disease course. Pearson's χ-test and odds ratios (ORs) were used for the purposes of statistical analysis, which was performed by SPSS, version 21.0 (Chicago, IL, USA). Additionally, the allelic and genotype frequencies of our Bulgarian healthy control subjects were compared with other populations.

Results

The mean age of the cases was 40.8 years (21-61), 35 women and 16 men. Familial MS was found in 20/51 (39.2%), 13 women and 7 men. In all of them there was maternal pattern of origin. Relapsing-remitting (RR) MS was found in 42 cases, relapsing-progressive (RP) in 5 and secondary progression (SP) after initial RR course was noted in 4. The mean age at the first symptoms was 30.4 (19-46) and the mean age of official diagnosis was 33.6 years (18-51). At the time of enrollment, the mean disease duration was 10.3 years (1-35) with a mean EDSS 3.68 (1.5-6.5). Past history of smoking and autoimmune diseases there was in 15/51 (29.4%) and 4/51 (7.8%), respectively.

The results are summarized in Tables 1-3.

Table 1: HLA-G allelic distribution

HLA-G	N	Aleles n (%)		p value	OR [CI]
		INS	DEL		
Controls	51	44 (43.1)	58 (56.9)	p>0.05	1.31 [0.75-2.28]
Cases	51	51 (50.0)	51 (50.0)		
Familial MS	20	24 (60.0)	16 (40.0)	p>0.05	0.51 [0.23-1.15]
Sporadic MS	31	27 (43.5)	35 (56.5)		

HLA-G – Human Leukocyte Antigen-G**, INS –** insertion**, DEL –** deletion**, OR –** Odds Ratio**, MS –** multiple sclerosis

Table 2: HLA-G genotypes distribution

HLA-G	N	Genotypes n (%)			p value	OR [CI]
		INS/INS	INS/DEL	DEL/DEL		
Controls	51	12 (23.6)	20 (39.2)	19 (37.2)	p>0.05	1.23 [0.50-3.00]
Cases	51	14 (27.4)	23 (45.2)	14 (27.4)		
Familial MS	20	8 (40.0)	8 (40.0)	4 (20.0)	p>0.05	0.4 [0.1-1.2]
Sporadic MS	31	6 (19.4)	15 (48.4)	10 (32.2)		

HLA-G – Human Leukocyte Antigen-G**, INS –** insertion**, DEL –** deletion**, OR –** Odds Ratio**, MS –** multiple sclerosis

Table 3: HLA-G genotype distribution by gender

		Case/control n (%)		p value (1-sided)	OR [CI]
	HLA-G	**Cases**	**Controls**		
males	**Ins/Ins**	0 (0)	6 (37.5)	**0.009**	**0 (0.000-0.0750)***
	Rest	16 (100)	10 (62.5)		
	N	16	16		
	Ins/Del	11 (68.8)	4 (25)	**0.016**	**6.600 (1.403-31.051)**
	Rest	5 (31.3)	12 (75)		
	N	16	16		
	Del/Del	5 (31.3)	6 (37.5)	0.500	0.758 (0.175-3.274)
	Rest	11 (68.8)	10 (62.5)		
	N	16	16		
females	**Ins/Ins**	14 (40)	6 (17.1)	**0.031**	**3.222 (1.063-9.768)**
	Rest	21 (60)	29 (82.9)		

N	35	35		
Ins/Del	12 (34.3)	16 (45.7)	0.232	0.620 (0.236-1.625)
Rest	23 (35.7)	19 (54.3)		
N	35	35		
Del/Del	9 (25.7)	13 (37.1)	0.220	0.586 (0.211-1.628)
Rest	26 (74.3)	22 (62.9)		
N	35	35		

*Statistically significant values are given in bold

Genotype distribution did not deviate from Hardy-Weinberg equilibrium among the healthy controls group. The comparison between cases and controls found no significant differences of the allelic and genotype distribution. When the patients group was divided according to the family history allele INS and genotype INS/INS were more frequent in familial than in sporadic cases and controls, but the differences were not significant.

Subsequently, the group of patients was divided according to the clinical characteristics to RR, RP and SP. Due to the small sample sizes (n≤5) of the groups of RP and SP patients, the p-values were not taken into consideration.

The sub-analysis by gender found significant difference between males and females (table 3). In males genotype INS/INS was found to be protective, whereas INS/DEL was associated with greater risk for MS. In females significant correlation with the risk for MS was found for the genotype INS/INS.

Discussion

HLA includes over 252 genes, located at 6p21 chromosome and grouped in two sub-regions – class I and class II. HLA I consists of two types molecules having different features – class Ia (classical - A, B, C) and class Ib (non-classical – E, F, G). In contrast to the classical class I (-A, -B and -C) molecules, HLA-G presents limited protein variability. Currently, 50 HLA-G alleles, encoding 16 functional proteins have been described and 2 null alleles.[7]

Seven HLA-G protein isoforms are result of alternative splicing of the primary transcript - four membrane-bound (HLA-G 1-4) and three soluble (HLA-G 5-7). HLA-G has unique structure, presenting a reduced cytoplasmic tail and capability to form dimers with enhanced LILR-mediated intracellular signaling.[8] It plays a significant role for establishing of the immune tolerance by Fas/FasL ligand mediated induction of apoptosis of CD 8^+ T cells and NK.[9,10]

Several single nucleotide polymorphisms (SNP) are described at DNA level, and those located in the promoter and 3'untranslated region (3'UTR) are frequently suggested to be functional. These polymorphic sites influence stability of mRNA and its turnover, mobility and splicing.[8,11] There are evidence that 14bp INS influences the mRNA stability and protein synthesis[12] and is also related to autoimmune diseases and some pregnancy-related conditions.[6,13] Two of them, 14bp DEL/INS and +3142C>G, have been proposed to be implicated in MS.[3] However they have not been studied intensively yet and there are few studies reporting conflicting results.

In series with 698 patients Kroner et al., reported no significant association with the susceptibility to MS, age of onset, severity or disease course in German population.[14]

A Polish case control study with 227 cases found significant correlation with three polymorphic sites of HLA-G.[15] On the background of similar distribution of 14bp INS/DEL in cases and controls the authors observed significant association with age at onset. The last was significantly higher in DEL/DEL homozygotes than in DEL/INS and INS/INS. Similar correlation they found for INS-positive vs. INS-negative cases.

In a recent series with 69 patients with relapsing-remitting MS, Rizzo and al., found that serum and cerebrospinal fluid levels (CSF) of soluble HLA-G are influenced by 14bp DEL/INS and +3142C>G polymorphisms.[4] The authors reported higher CSF levels of sHLA-G in MRI inactive than the cases with active disease. The combined analysis of 14bp INS/DEL and +3142C>G polymorphisms revealed the highest serum and CSF concentrations of sHLA-G in the high producers cases (DEL/DEL and +3142 C/C genotype) whereas the lowest titers were found in low producers (INS/INS and G/G genotype) both in active and inactive disease. This finding implicates

a possible role of HLA-G in MS on one hand, and that the release of sHLA-G in serum and CSF may be regulated not only by local environment but also by these two polymorphisms. There was no correlation of these polymorphisms with disease duration and disability.

Wiendl et al. assessed 11 brain specimens and cerebrospinal fluids of MS patients which compared to specimens from other neurological controls.[16] They reported significantly higher over-expression of HLA-G in the transition zone of chronic active plaques and in perilesional microglia cells than in healthy controls and overexpression of its receptor ILT-2 on macrophages and microglia. Moreover the authors observed stronger upreglation of HLA-G mRNA after Th1 than Th2 inflammatory stimulation. Another important finding represents the higher cerebrospinal fluid concentrations of soluble HLA-G in MS than in other neurological controls.

Fainardi et al., showed significantly higher cerebrospinal fluid levels of sHLA-G and lower levels of anti-apoptotic sFas in patients with RR-MS versus controls with other inflammatory and non-inflammatory neurological diseases in Italian population.[17] The analysis according to clinical and MRI activity showed increased levels of HLA-G and decreased Fas levels in MRI-inactive patients.

On one hand, sHLA-G may suppress the autoimmunity in MS acting as anti-inflammatory molecule. This effect is mediated by Fas/FasL-mediated apoptotic elimination of the activated $CD8^+$ T cells and natural killers. Additionally it shifted Th1/Th2 balance toward Th2 through the inhibition of $CD4^+$ Th1 cells proliferation and increase of IL-10 production.[5]

On other hand, HLA-G polymorphisms might determine the serum and CSF levels of sHLA-G irrespectively of the local inflammation. It is thought that the production of sHLA-G from HLA-G^{pos} T_{reg} is decreased in the patients with genotype INS/INS (low producers) and increased in INS/DEL and DEL/DEL genotypes (high producers).[18] This observation led to the hypothesis that the individuals with lower sHLA-G levels are at greater risk for MS than those with normal or higher levels, independent of the disease activity.

The present study found no significant discrepancy of allelic and genotype distribution between cases and controls. The frequency of INS allele INS/INS genotype in familial cases and sporadic cases were 60% vs. 44% and 40% vs. 19%, respectively. Similar result was observed when familial cases were compared with the controls (INS 60% vs 43%, INS/INS 40% vs. 24 %).

The analysis by gender revealed significant difference between males and females (table 3). A significant correlation was found for genotype INS/INS in females. Surprising and reciprocal to the literature results were found in males. Genotype INS/DEL (high producers) was associated with higher risk, whereas INS/INS (low producers) was found to be protective. In our opinion this finding should be interpreted with a caution.

An important drawback of our study is the small sample size due to financial limitations. In this light the lack association may reflect a type II error. This limitation might be overcome by multicenter collaboration in the future in which we may participate with the established MS-DNA bank.

Conclusion

Based on the results, the HLA-G 14bp INS/INS is likely to be involved in familial MS in our population and is associated with MS in females. The finding in males should be interpreted with a caution. The results warrant additional studies with international collaboration and larger sample size.

Conflict of interest: The authors have no conflict of interest.

Disclosure:

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