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HLA Class I & II Alleles in Multiple Sclerosis patients from Puerto Rico

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Abstract

Multiple Sclerosis (MS) is a complex disease where genetic and environmental factors have been implicated. The onset of symptoms occurs in individuals from twenty to fifty years of age, producing a progressive impairment of motor, sensory and cognitive functions. MS is more frequent in females than in males with a ratio of 4:1. The prevalence of the MS varies among ethnics groups such as Europeans, Africans and Caucasians. The estimated prevalence of MS in Puerto Rico is 42 for each 100,000 habitants, which is more than the prevalence reported for Central America and the Caribbean. In spite of this prevalence, the genetic component of MS has not been explored in order to know the alleles' expression of Puerto Rican MS patients and compare it with the allele expression in other ethnic groups. Thirty-five patients and 31 control subjects were genotyped. The allele frequencies expressed in this sample were similar to those expressed for Puerto Ricans in the National Marrow Donor Program Registry (n=3,149). The most prevalent alleles for MS patients were HLA-DRB1*01 and *03. HLA-DQB1*04 was the most frequent in the control group and HLA-A*30, in MS patients. These findings are in agreement with published data. HLA-DQB1*04 was a marginal protector in this sample and this role has not been described before. The accuracy of the results is limited due to the sample size. After performing a statistical power analysis it showed that by increasing the sample the values would be significant.

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Index words

HLA; alleles; genotyping; multiple; sclerosis; Puerto Rico

INTRODUCTION

Multiple Sclerosis (MS) is considered a complex disease where genetic and environmental factors have been implicated. Approximately two million people worldwide and more than 400,000 persons in the United States are living with MS. Generally, the onset of symptoms occurs in individuals from twenty to fifty years of age, producing a progressive neurological dysfunction. The most common presenting symptoms are optic neuritis, diplopia, paresthesias, fatigue, difficulties in motor coordination, and cognitive dysfunction (1). The incidence and prevalence of MS is higher in latitudes north and south of the Equator (2, 3). In Puerto Rico the estimated prevalence of MS is 42/100,000 habitants, according to the MS Epidemiological Study and more frequent in females than in males with a ratio of 4:1, in contrast to the prevalence reported by GEEMAL for Central America and the Caribbean that range from 4.4 - 30/100,000 habitants (4, 5).

The exact cause of the disease is not known. Studies analyzing brain necropsies of MS patients; based on the animal model of the disease - the Experimental Autoimmune Encephalitis- observing an increase of proinflammatory cytokines and a decrease in the activity of regulatory T cells and by the identification of antibodies produced against the components of the myelin in the cerebrospinal fluid have demonstrated that the immune system is a major player in the etiology of MS (6-9). MS produces a severe demyelinization of the central nervous system, with axonal and neuronal loss (10, 11).

The first reports of associations between genetic markers and autoimmune diseases were published in the 1960’s, identifying the HLA locus as a critical region in the predisposition to the disease (12, 13). Several genes have been associated to the genetic susceptibility. Such genes interactions, in addition to environmental factors, could produce the inflammatory response that results in the CNS white and gray matter lesions. Other genes involved are TCR, IFN, VDR, TNF, IL-1, IL-2, IL4R and, the APO4 allele, among others (14, 15). The development of new technologies such as the genome-wide association studies (GWAS) have provided an excellent approach to understanding the relationship of several genes by genotyping common known single nucleotide polymorphisms (SNPs) from the HapMap Project. A comparison between two large groups is made and expressed in odds ratio, based on the frequency of the alleles in both groups. These studies have confirmed 16 loci with genome-wide significance. Several of the common risk variants are present on genes involved in immunologic functions and associated to autoimmune diseases (16).

Family studies have confirmed that susceptibility to MS is at least partly familiar (17). The risk of MS occurrence in monozygotic twins was 31%, whereas in dizygotic twin was only 5%. The risk of a sibling or a parent of an affected person was 3 to 4%, compared to the risk of the general population of 0.1% (18). Sadovnick (1996) showed that not all MS patients express the HLA-DRB1*15 alleles associated to MS (19). Studies performed in other geographical regions showed that alleles described to be present in MS patients were present

in the vast majority of samples analyzed; they also described differences in allele expression in other ethnic groups and geographical regions shown in Table 1: Diversity of HLA Alleles' Expression in Multiple Sclerosis.

HLA Class I and Class II alleles' expression of a group of Puerto Rican RRMS patients and control subjects were analyzed and compared findings to the HLA expression published for other ethnic groups and geographical regions, in order to: 1) determine their haplotype and see if it is similar to the haplotypes described for other ethnic groups and geographical regions, and 2) seeking for presence of new specificities that could favor a predisposition in developing MS in an attempt to understand the higher prevalence of MS in Puerto Rico.

MATERIAL AND METHODS

Patients and control subjects

Patients were recruited between May and November 2007 from a cohort of MS patients, undergoing follow up visit at the San Juan MS Center. The data set comprised 35 patients diagnosed with definite remitting-relapsing multiple sclerosis (RRMS); self reported as Puerto Ricans and after a neurologist evaluation, according to McDonald criteria (20) and once evaluated and met the inclusion criteria. The 31 control subjects were individuals without a definite MS diagnosis, inflammatory diseases or malignancies; matched ethnically and geographically, for age, gender, and social background. They were recruited from other patients visiting the San Juan MS Center, clinical laboratories, and university students. All study participants were self-reported as Puerto Ricans. IRB approval and informed consent from all study participants was obtained.

DNA sample collection

Two tubes of peripheral blood from patients and controls were obtained by phlebotomy, following the procedures established by accrediting agencies. DNA was extracted from peripheral blood using commercial methods (QIAamp, QIAGEN). DNA concentration was adjusted to 20μg/μl with a purity of OD260/280 of 1.65 to 1.80. The DNA samples were kept frozen at the PI laboratory, until mailed to the National Human Genome Center at Howard University, Washington, DC.

HLA Genotyping

MHC HLA Class I (HLA-A and B) and Class II (HLA-DR and DQ) genotyping was performed using a commercial kit (LABType SSO Typing Tests, One Lambda Co. LA, California). HLA alleles' detection was done using a non-radioactive PCR-SSOP (polymerase chain reaction-based sequence specific oligonucleotide probe) reverse line-blot assay, and analyzed in a LABScan™100. Briefly, target DNA was PCR-amplified using specific primers for the HLA Class I (HLA-A and B) and Class II (HLA-DR and DQ) locus. The PCR product was denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A flow analyzer identified the fluorescent intensity of phycoerythrin (PE) on each microsphere. The assignment of the HLA typing was based on the reaction pattern compared to patterns associated with published HLA gene sequences.

Statistical Analysis

An epidemiological profile of the participants was performed using frequencies distributions and descriptive statistics (mean, standard deviation, and percentiles). Graphs and Box plot were used to better understand the similarities and differences in the two study groups. To describe the frequencies of the alleles by type of participant (MS and control) a contingency Table was utilized. To determine significance difference among the alleles frequency by type of participants, the odds ratio was estimated with 95% confidence using a logistic regression model. In three loci some alleles had very small frequency (<6), so they were grouped in a category called “others” and this category was taken as the reference group for computing odds ratio (OR).

RESULTS

Mean age of MS patients was 46.7 years. Females mean age was 46 years SD (9.7) and males mean age was 49.3 years SD (9.4). Female to male ratio was 4:1. Mean age of control subjects was 34.6 years; females mean age was 41 years SD (18.2) and male mean age was 34.5 years SD (15.5). Female to male ratio was 3.43:1 (see Table 2). Sixty-six individuals (35 MS patients and 31 control subjects) were genotyped for HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1 locus. Allele frequencies were analyzed at a resolution corresponding to serological specificities and, compared to data for Puerto Ricans provided by DNA data bank of the National Marrow Donor Program (NMDP), in order to demonstrate that alleles found in this research were representative of the Puerto Rican population.

Alleles’ frequencies

Seventeen different HLA-A alleles were observed in the samples genotyped: The most frequent alleles were *02, *24 and *03. Twenty- six different HLA-B alleles were observed in the samples genotyped: The most frequent alleles were *44, *35, *15, *41 and *08. Thirteen different HLA-DRB1 alleles were observed in the samples analyzed: The most frequent alleles were *13, *04, *03, *07 and *15. Five different alleles were observed in the samples genotyped for HLA-DQB1 locus: The *03 allele was the most frequently observed followed by *02, *06, *05 and *04 (see Table 3).

HLA-A, HLA-B, HLA-DRB1, and HLA-DRB1 alleles’ frequencies and the risk for MS

To determine whether the alleles expressed in the study sample influenced the magnitude of risk for MS, logistic regression was used to examine the effect of HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 alleles compared to controls, to yield odds ratios with 95% confidence intervals. For HLA-A, the results after the analysis were: the OR for HLA-A *30 was 7.2 (95%CI: .74, 70.2) times the odds of having MS in participants classified in the category “others”. This excess was marginally significant (p-value= 0.089). The rest of the alleles did not show statistical evidence of significant increments (P-value>0.1). For HLA-B, the results were: the odds of having MS in participants with allele *15 was 3.17 (95%CI: 0.58, 17.15) times the odds of having MS in participants classified in the category “others”. However, this excess was not significant (p-value=0.181). The odds of having MS in participants with allele *35 was 1.35 (95%CI: .33, 5.55) times the odds of having MS in participants classified in the category “others”. However, this excess was not significant (p-

value=0.671). The odds of having MS in participants with allele *41 was 1.20 (95%CI: 0.23, 6.09) times the odds of having MS in participants classified in the category “others”. However, this excess was not significant (p-value=0.821). The odds of having MS in participants with allele *44 was 0.75 (95%CI: 0.19, 2.87) times the odds of having MS in participants classified in the category the “others”. However, this reduction was not significant (p-value=0.697) (see Table 4).

In summary, there were not significant differences (p-value>0.05), the higher increments were observed in the allele *15 with respect to the category “others”. The odds of having MS in participants with allele HLA-DRB1 *01 was 11.0 (95%CI: 1.1, 109.7) times the odds of having MS in participants classified in the category “others”. This excess was statistically significant (p-value= 0.041). The odds of having MS in participants with allele *03 was 4.3 (95%CI: 0.97, 19.1) times the odds of having MS in participants classified in the category “others”. This excess was marginally significant (p-value=0.054). The rest of the alleles did not showed statistical evidence of significant increments (P-value>0.1). For HLA-DQB1, the results were: the odds of having MS in participants with allele *04 was 0.23 (95%CI: 0.05, 1.09) lower than the odds of having MS in participants with allele *02. This excess was marginally significant (p-value=0.066). The rest of the alleles did not show statistical evidence of significant increments (P-value>0.1).

DISCUSSION

Sixty-six samples from MS patients and control subjects were successfully genotyped. Mean age of MS patients and female to male ratio was similar to results obtained in the Epidemiological study conducted by the Puerto Rico MS Foundation (4). Analysis of data generated in present study was performed at low resolution, in order to compare the results to statistics for Puerto Ricans, provided by the National Marrow Donor Program. Most frequent alleles for HLA-A locus were*02, *24 and *03; for HLA-B locus, most frequent alleles were *44, *35, *15, *41 and *08; for HLA-DRB1 locus, most frequent alleles were *13, *04, *03, *07 and *15, and for HLA-DQB1 locus, *03 allele was most frequently observed, followed by *02, *06, *05 and *04. Comparison of alleles’ frequencies reported by the NMDP databank for Puerto Ricans and alleles expressed in the control group of this study demonstrated a correspondence of alleles expressed in both groups for HLA-A, HLA-B and HLA-DRB1 locus. Some findings of this study are in agreement to the findings published in literature. Smestad et al. (2007) were not able to identify a significant contribution of the HLA-A alleles in association to the clinical phenotypes of MS (21). This group, in an unpublished data, observed that the presence of HLA-A*02 decreased the risk of MS, significantly (22).

In this study, most frequent allele observed in the control group was HLA-A*02. This allele is also frequent in the data provided by the NMDP. This study also found that HLA-DRB1*01 was the most represented allele in MS patients. DeLuca et al. (2007) provided evidence that HLA-DRB1*01 is not a protective allele, as stated by other researchers (21, 23). This allele acts as an independent modifier of the disease progression because they found that HLA-DRB1*01 was more frequent in patients with benign multiple sclerosis than in patients with the malignant form. Brynedal et al (2007) and Barcellos et al. (2006), in two

studies performed with Sweden, Sicilian and Spanish families, the authors implicated the HLA-DRB1*03 allele in the risk of MS (24; 25). The HLA-DRB1*03 allele was the second most represented in MS patients in the present study. Dean et al. (2007) points to HLA-DRB1*03 and *04 alleles as influencing the risk of the disease in Malta. It is suggested that HLA-DRB1*03 may be involved in MS as a secondary MS marker (26). The present study found that HLA-DQB1*04 was the allele less represented in MS patients. This allele might confer a protective role against the disease. This has not been described before in literature as an allele implicated in susceptibility or resistance to MS. More studies should be done in the Puerto Rican population to confirm these results, because the 95% confidence intervals were large.

This study represents the first genetic study performed with a group of MS patients in Puerto Rico. The prevalence on the disease in Puerto Rico is four times higher than numbers reported for the Tropics by the MS International Federation (MSIF) and GEEMAL (5). Although the high prevalence of MS; there is no information about the genetics of the disease in Puerto Rico. Other genetic studies in the Island have shown that rare genetic conditions are more common in specific regions of the Island than worldwide. It is necessary to perform more studies to determine the relationship between HLA alleles, and the clinical presentations and severity of the disease. This study results show the existence of alleles that predispose and confer resistance to the disease in the Puerto Rican MS patients, which are statistically significant; others are marginally significant due to the small size of the group. A Statistical Power Analysis performed showed that an increase in the sample number would show the same results with a decrease in the CI (see Table 5). The genetic profile of the Puerto Rican population provides a unique model to study the immunobiology of Multiple Sclerosis

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Table 1

Diversity of HLA alleles' expression for MS patients in different geographical areas and ethnic groups on published data.

Diversity of HLA Alleles Expression in Multiple Sclerosis	
Northern Europeans (Australia, Scandinavia, New England)	DR15 (DRB1*1501-DRB5*0101)
Africans	A low frequency of DRB1*1501
African American	Great variability of the locus DRB1 DRB1*1501, DRB1*1503 and DRB1*0301
Martinique	DRB1*07; and the most common subtype of DRB1*15 was *1503, not *1501
United States	HLA-DRB1 and the DR2 haplotype (DRB1*1501-DQB1*0602)
Mexicans	Two Class II novel associations DRB1*0403, DRB1*0802
Asiatic Indians and Afro-Caribbean	DRB1*1501, DQA1*0102, DQB1*0601 Low association with MS
Afro-Brazilian	DQB1*0602 genetic susceptibility
Malaga, Spain	DRB1*1501, DQA1*0102 and DQB1*0602

Table 2

Epidemiological Data for MS Patients and Control Subjects.

Epidemiological Data for Study Subjects				
	Mean age (years)	**Females mean age (years)**	**Males mean age (years)**	**Female to Male Ratio**
Control subjects	34.6	41 SD (18.2)	34.5 SD (15.5)	3.43:1
MS patients	46.7	46 SD (9.7)	49.3 SD (9.4)	4:1

Table 3

Most Frequent Alleles Express in Sample.

Most Frequent HLA Alleles	
HLA-A locus	*02, *24, *03
HLA-B locus	*44, *35, *15, *41, *08
HLA-DRB1 locus	*13, *04, *03, *07, *15
HLA-DQB1 locus	03, *02, *06, *05, *04

Table 4

Summarizes the most relevant findings of the HLA genotyping MS patients as a function of Odds Ratio (OR) and compare the numbers to the roles given by the researchers in other ethnic groups.

Summary of findings of Preliminary Study			
Allele	**OR* (95%Cl)**	**p <0.1**	**Comments**
*HLA-A *30*	7.2	0.089	Most frequent in MS patient - marginally significant Amirzargar et al. (2005) Optical Neuritis; Bitti et al. (2001)
*HLA-A *02*	0.7	0.552	Smestad et al. (2007), Decreased risk of MS Bergamaschi et al. (2010); Protective allele
*HLA-DRB1 *01*	11.0	0.041	DeLuca et al. (2007) MS benign disease
*HLA-DRB1 *03*	4.3	0.054	Barcellos et al. (2006); Brynedal et al. (2007); Dean et al. (2007) Risk of MS
*HLA-DQB1 *04*	0.23	0.066	Marginally significant; Protective role; not described before