

HLA A, B, Cw, DRB1 and DQB1 Alleles in Multiple Sclerosis Patients in India

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ABSTRACT Multiple sclerosis (MS) is a clinically heterogeneous demyelinating disease and an important cause of acquired neurological disability. MS has been reported from different regions of India and its infrequency has been attributed to have genetic implications. We analyzed the HLA A, -B, -Cw, -DRB1 and DQB1 allele associations in 23 clinically definite MS patients and compared with 146 clinically normal healthy controls. The HLA A, B, Cw, DRB1 and DQB1 alleles were identified the genomic DNA extracted using commercially procured DNA extraction kit (Qiagen kit), HLA A*, HLA B*, HLA Cw*, HLA DRB1*, and HLA DQB1* alleles were identified by PCR-SSOP typing using the commercially procured kits (Dynal or Innolipa). The study revealed a significant increase in HLA A*11:01:01 (pvalue=0.03), B*39:01:01:01 (OR=13.8; pValue=0.0006), Cw*07:01:01 (pValue=0.03), DRB1*15:01:01: (pValue=0.002) and DQB1*02:01:01 (OR=9.6; pvalue=9.57E-06) while a significant decrease in HLA A*24:02:01:01 (pvalue=0.09), B*40:06:01:01 (P value=0.06), Cw*03:02:01 (P value 0.002), DRB1*10:01:01 (pValue=0.06) and DQB1*06:01:01 (pvalue=0.06). Our study reveals that there is a population specific HLA allele genetic susceptibility or protection to MS in different populations reported in literature.

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system caused by interplay of environmental and genetic factors. The only genetic region that has been clearly demonstrated by linkage and association studies to contribute genetic susceptibility is the HLA system. The association between MS and class II alleles of MHC in particular HLA DRB1*1501 and its associated haplotypes have been has reported from word over in majority of Caucasian and North European populations. Further, MS has been reported from different regions of India (Mathew et al. 1971; Singhal and Wadia 1975; Chopra et al. 1980; Gauri-devi et al. 1982; Jain and Maheswari 1985; Singhal 1987; Pandit et al. 1993) as well. The rough estimate for the prevalence of MS would be 1.33 per 100,000 individuals in Mumbai. Earlier HLA B12 associations in MS patients of highly inbred Parsi population and

HLA A11, B16, Cw7 and DRB15 in non-Parsi MS patients from Mumbai have been reported (Bharucha et al. 1988; Wadia and Bhatia 1990). However, a consistent HLA association has not been evaluated in other communities from Mumbai. Considering the HLA diversity of India, we included clinically definite MS patients group belonging to non- Parsi from Mumbai in this study.

MATERIALS AND METHODS

Patients and Controls

Clinically definite 23 MS patients defined according to Posers criteria (Poser et al. 1983) attending Bombay hospital Institute of Medical sciences were included after obtaining ethical consent. A detailed evaluation of patient history, identified clinical variables, disease severity, age at onset, initial clinical manifestations and informed consent were recorded for every patient. The MS was diagnosed after through neurological examinations for spinal cord and CNS involvement, sites of lesion, course of disease, Kuntzkes extended disability status, family history etc. CSF analysis for IgG, visual evoked potentials, brain stem auditory evoked poten-

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tials, somatosensory evoked potentials, MRI of brain and/or spinal cord were all done in all patients to confirm the MS diagnosis. Further investigations were performed to exclude other connective tissue disorders. One hundred and forty six ethnically age and sex matched normal individuals studied for their HLA in the tissue typing laboratory of Bombay hospital without the clinical disease symptoms were compared as controls.

HLA Typing

The HLA A, B, Cw, DRB1 and DQB1 alleles were identified the genomic DNA extracted using commercially procured DNA extraction kit (Qiagen kit), HLA A*, HLA B*, HLA Cw*, HLA DRB1*, and HLA DQB1* alleles were identified by PCR-SSOP typing using the commercially procured kits (Dynal or Innolipa; Invitrogen) for the high resolution HLA alleles as per 2010 April Nomenclature. The statistical analysis Such as odds ratio, etiological fraction, Chi-square, and level of significance (p value) for the ELISA values were estimated as described earlier (Kankonkar and Shankarkumar 2008).

RESULTS

The HLA A*, B* and Cw* alleles identified are presented in the Table 1. A significant increase in HLA A*11:01:01 (OR=2.6; pvalue=0.03), B*39:01:01:01 (OR=13.8; value=0.0006) and Cw*07:01:01 (OR=5.46; value 0.03), while a significant decrease in HLA A*24:02:01:01 (OR=0.42; pvalue=0.09), B*40:06:01:01 (OR=0.27; value=0.06) and Cw*03:02:01 (OR=0.08; value 0.002) was observed. The HLA alleles A*32:01:01, A*68:01:01, B*08:01:01, B*13:01:01, B*49:01:01, Cw*04:01:01:01 and Cw*06:02:01:01 did not show significance after applying Bonferroni's correction. The HLA DRB1* and DQB1* alleles identified are presented in Table 2. A significant increase in HLA DRB1*15:01:01:01 (OR= 16.5; pValue = 0.002) and HLA DQB1*02:01:01 (OR=9.6; pvalue= 9.57E-06) while a significant decrease in HLA DRB1*10:01:01 (OR= 0.154; pValue=0.06) and HLA DQB1*06:01:01 (OR=0.376; pvalue=0.06) was observed.

The HLA alleles DRB1*03:01:01:01, DRB1*07:01:01:01, DRB1*11:01:01, DRB1*14:01:01, DRB1*15:06, DRB1*15:08, DQB1*03:03:02, DQB1*05:02:01 and DQB1*

Table: 1 HLA A, HLA B and HLA Cw alleles in Mumbai MS patients

HLA allele	MS pts AF(%) N = 23	Controls AF(%) N = 146	OR	EF	PF	p value
A*11:01:01	23.90	13.00	2.605	0.14		0.032
A*24:02:01:01	10.80	19.80	0.421		0.12	0.097
B*08:01:01	8.60	3.42	2.863	0.05		0.08
B*39:01:01:01	4.30	0.34	13.800	0.03		0.0068
B*40:06:01:01	4.30	13.00	0.270		0.09	0.069
B*49:01:01	4.30	0.68	6.857	0.03		0.03
		N = 27				
Cw*03:02:01	4.30	25.92	0.088		0.29	0.002
Cw*07:01:01	15.20	3.70	5.468	0.12		0.034

AF (%) Allele frequency in percentage OR= Odds ratio EF=Etiological fraction PF=Preventive fraction

** Significant P value

Table 2: HLA DRB1 and DQB1 allele associations in Mumbai MS patients

HLA allele	MS pts AF(%)	Controls AF(%)	OR N = 18	EF N = 45	PF	p value
DRB1*15:01:01:01	21.70	2.20	16.153	0.19		0.0023**
DRB1*15:06	10.00	16.66	0.500			
DRB1*15:08	15.00	0.00	6.000	0.13		0.279
DRB1*10:01:01	2.10	11.30	0.154		0.09	0.069
DQB1*02:01:01	36.11	5.55	9.608	0.32		9.51E-06**
DQB1*05:01:01	16.66	4.44	4.3	0.12		0.021
DQB1*06:01:01	13.88	30	0.376		0.17	0.06

AF (%) - Allele frequency in percentage OR=Odds ratio EF=Etiological fraction PF=Preventive fraction ** Significant P value

06:01:02 did not show significance after applying Bonferroni's correction

In our MS series studied most of the patients were in the age group of 21-30 years, while the male: female ratio was 6:17. The clinical sites of involvement among our MS patients revealed spinal cord (78.2%), eyes (82.6%), Brainstem (34.7%), cerebellum (34.7%) and cortical (21.7%) to be involved. Further it was observed that 95.65% of our MS patients had a relapsing remitting course of disease. The MRI of brain showed 65.2% abnormality while MRI of spinal cord showed 69.5% abnormality among the MS patients. The CSF IgG levels were positive in 83.3% of the MS cases. The recorded visual evoked potentials (91.3%), brainstem auditory evoked potential (17.3%) and somatosensory evoked potential (26%) were abnormal in MS patients studied. Treatment with N methyl prednisolone was followed in 91.3% of the patients while 13% had to be given Interferon and Azathioprine as the mode of treatment. However none of the patients developed side effects during the treatment.

DISCUSSION

Relatively large numbers of studies have been done on the associations of HLA antigens in Multiple sclerosis (Kankonkar et al. 2003). Consistent HLA DR2, its subtype DRB1*1501 and associated haplotype associations along with other genetic markers have been reported from populations world over (Kikuchi et al. 2002; Healy et al. 2010). However, studies from Indian MS patients are few (Bansil et al. 1997; Syal et al. 1999). Majority of the HLA population studies in MS have focused on Caucasians, Australians, Chinese, Sardinians, Japanese, Jewish, Swedish, Turks and canary Island where the predisposition of the disease has been consistently associated with DRB1*1501 and its associated haplotype DRB1*1501-DQA1*0102-DQB1*0602 (Lopez-Larrea et al. 1990; Kwon et al. 1999; Allen et al. 1994; Fogdell-Hahn et al. 2000). However positive associations with HLA DR4 have also been reported in Sardinians and other Mediterranean populations (Marrosu et al. 1998; Coraddu et al. 1998). Moreover DR1, DR7, and DR11 have been found to be protective in several populations. Further much limited inconsistent studies have been reported for HLA class I allele associations in Italian, Japanese, Swedish, Russian and Spanish MS patients

(Bitti et al. 2001). Recently, HLA gene analysis in clinically well-characterized Arab populations with MS, showed DRB1*0301 allele in Muslims ($P(\text{Bonferroni})=0.004$, odds ratio (OR)=3.07), and negative association in Christian Arabs ($P(\text{Bonferroni})=0.01$, OR=0.12), with similar results obtained for HLA-DQB1*0201. HLA-B*52 was negatively associated with MS only in Muslims ($P(\text{Bonferroni})=0.01$, OR=0.03) and the shows population-specific contribution of the DRB1*0301-DQB1*0201 haplotype to disease susceptibility (Benedek et al. 2010).

In genotyping studies suitable for HLA-DRB1 allele meta-analysis, showed that HLA-DRB1*15 was associated with risk of MS in the combined group (308 cases and 407 controls; OR 1.39) while the HLA-DRB1*09 and HLA-DRB1*0901 alleles were protective meta-analysis suggests that HLA-DR2/DRB1*15 are also associated with risk of MS in the Chinese population but less strongly than in Western MS populations, whereas HLA-DR9 alleles appear to confer resistance (Qiu et al. 2010).

In the present study we have found the associations for HLA A*11:01:01, B*39:01:01:01, Cw*07:01:01, DRB1*15:01:01:01 and DQB1*02:01:01 while protection for in HLA A*24:02:01:01, B*40:06:01:01, Cw*03:02:01, DRB1*10:01:01 and DQB1*06:01:01, in our MS patients. The observations of different allele and haplotype associations among MS in various populations emphasize that there is a population specific HLA allele genetic susceptibility or protection to MS in different populations reported in literature. Further the immunological mechanisms are known to play crucial roles in MS pathogenesis as well has to be elucidated in a population specific manner in order to understand the underlying immunological mechanisms.

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