Original Article

CD6 gene polymorphism rs17824933 is associated with multiple sclerosis in Indian population

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Abstract

Background: Multiple sclerosis (MS) prevalence has increased worldwide. The known genetic association for MS in the west has not been studied in detail in nonwhite populations and particularly Indians. **Objective:** The objective of this study was to evaluate some known genetic variations outside the major histocompatibility complex (MHC) region associated with MS in patients of Indian origin. **Materials and Methods:** We investigated 10 gene-associated single nucleotide polymorphisms (SNP's) outside the MHC region in 300 patients and 720 unrelated controls. Genotyping was performed on an ABI7500 real-time polymerase chain reaction genotyping platform using predesigned TaqMan SNP genotyping assays. **Results:** CD6 gene associated SNP (rs17824933) showed significant association with MS ($P = 4.2 \times 10^{-5}$, odds ratio [OR] = 2.24, confidence interval (CI) = 1.51–3.33). A modest association was also noted for TMEM39A rs1132200 ($P = 0.023$, OR = 1.41, CI = 1.05–1.91) and IL2RA rs2104286 ($P = 0.04$, OR = 1.3, CI = 1.006–1.67). In the remaining SNPs, the allele frequencies were overexpressed in patients when compared to healthy controls. **Conclusion:** Our data illustrate the similarity in risk association between Indian and European populations for MS.

Key Words

Genetic susceptibility, Indian, multiple sclerosis, single nucleotide polymorphism, South Asians

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Ann Indian Acad Neurol 2016;19:491-494

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system. Both genetic and environmental factors are likely to contribute to the etiology of the disease.[1,2] The contribution of genetics to MS is supported by many reports showing familial aggregation of the disease, high concordance rates among twins, and an increased risk among relatives of patients with MS.[3] Genetic susceptibility for MS is significantly associated with the genes associated with the major histocompatibility complex (MHC).[1] It is now clear that HLA-DRB1 * 15:01 is the principal risk allele and the haplotype exerting greatest effect on risk is HLA-DRB1 * 15:01-DQA1 * 01:02-HLA-DQB1 * 06:02. Decades after this discovery genetic variants were identified outside the MHC region which was associated with MS. These include IL7R, IL2RA, CLEC16A, CD226, GPC5, EV15, TYK2, CD58, TNFRSF1A, IRF8, and CD6, since then several genome-wide association studies have been performed which identified a number of common genetic variations that confer modest risk for MS.[4-13] To date, 110 variants have been identified that influence susceptibility to MS among European populations.[14] These variants consistently implicate genes associated with immunological processes lying in regulatory rather than coding regions and are frequently associated with other autoimmune diseases.[2]

Among South Asians, the study of genetic susceptibility for MS has been limited. For Indians, the strong association with HLA-DRB1 * 15:01 has been recently established.[15] An

Access this article online	
Quick Response Code:	Website: www.annalsofian.org
	DOI: 10.4103/0972-2327.192384

How to cite this article: D'Cunha MA, Pandit L, Malli C. CD6 gene polymorphism rs17824933 is associated with multiple sclerosis in Indian population. Ann Indian Acad Neurol 2016;19:491-4.
Received: 20-01-16, **Revised:** 15-02-16, **Accepted:** 01-03-16

earlier study of 197 patients and controls had shown nominal association with two variants, rs12708716, and rs763361 related to CLEC 16A and CD226 genes, respectively.[16] A replication study of 110 European risk variants has been recently conducted among Indians.[17] In this study, two-thirds of the tested variants (72/109) showed over-representation of European risk allele in South Asian cases ($P < 0.0003$). In the remaining, the most associated variant was rs7318477 which maps close to TNFSF13B, the gene for B-cell related protein B-cell activating factor. In this study, we have used a new and larger data set of 300 MS patients and 720 healthy controls and evaluated the role of 10 single nucleotide polymorphisms (SNP's) outside the MHC region which are known to be associated with MS in Europeans.

Materials and Methods

Sample collection

Patients were obtained from the Mangalore demyelinating disease registry.[18] All consecutive patients selected were diagnosed by McDonald criteria.[19] Unrelated controls were spouses or friends who belonged to the same caste and geographical region as patients. The latter was done to match for ancestry and to reduce the risk of confounder effect.[20] Clinical characteristics and demographic features were recorded [Table 1]. This work was done in compliance with the Declaration of Helsinki. The study was approved by the Institutional Ethics Committee. Informed consent was obtained before blood draw.

Single nucleotide polymorphism genotyping

A total of 10 nonMHC SNPs were selected for genotyping [Table 2]. SNP genotyping was performed on an ABI7500 real-time polymerase chain reaction (PCR) genotyping platform using predesigned TaqMan SNP genotyping assays (Applied Biosystems Inc., Foster City, CA, USA) as described earlier.[16] In short, 4 μl of normalized genomic DNA (5 ng/ul) was aliquoted to the bottom surface of a MicroAmp® Optical 96-Well Reaction Plate. The DNA sample was dried down completely by evaporation. Each PCR contained 20 ng DNA, 12.5 μl Taq Man Universal PCR Master Mix (×2), 1.25 μl SNP genotyping assay (×20), 11.25 μl DNase-free water. The PCR conditions were as follows: 60°C for 1 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. After PCR amplification, endpoint plate read was performed using an Applied Biosystems 7500 real-time PCR system. The Sequence Detection System (Applied Biosystems 7500 software v2.0.6) software uses the fluorescence measurements made during the plate read to plot fluorescence (*Rn*) values based on the signals from each well. The plotted fluorescence signals indicate which alleles are present in each sample.

Statistical methods

The genotyping success rate, Hardy–Weinberg equilibrium, marker heterozygosity, and data analysis were established using PLINK 1.07 statistical software (Center for Human Genetic Research, Massachusetts General Hospital, Cambridge Street, Boston).[21]

Results

A total of 300 cases and 720 controls were analyzed in this study. All 10 SNP's were in Hardy–Weinberg equilibrium. The average genotyping success rate across the markers was 98%. In this study, the most significant association was seen with rs17824933 ($P = 4.2 \times 10^{-5}$, odds ratio [OR] = 2.24, 95% confidence interval [CI] =1.51–3.33). Two other SNPs which showed a modest association with MS in this study are TMEM39A rs1132200 (P = 0.023, OR = 1.41, CI = 1.05–1.91) and IL2RA rs2104286 ($P = 0.04$, OR = 1.3, CI = 1.006–1.67). For the remaining SNPs, the risk allele frequency was over-expressed in cases as compared to healthy controls [Table 3]. Overall, the risk allele frequency was similar between Indian and white populations.

Discussion

The SNP's genotyped in this study were selected because of their known risk-modifying effects for MS in white populations. The lower prevalence of MS in nonwhite populations has limited the power of studies evaluating genetic susceptibility. Despite these limitations, recent studies in nonwhite populations have shown similarity for MS risk variants that have been identified in European populations. These include African Americans[22,23] and South Asian Indians.[16,17]

In this study, CD6 associated SNP showed the strongest association ($P = 4.2 \times 10^{-5}$, OR = 2.24, 95% CI = 1.51–3.33) among the variants studied. CD6 is a cell surface scavenger receptor involved in T-cell activation and proliferation, as well as in thymocyte differentiation. CD6 has recently been identified and validated as a risk gene for MS, based on the association of an SNP, rs17824933, located in intron 1.[11] In accordance with results from Spain[24] and European groups,[25] our results have revealed that rs17824933 is significantly associated with an increased disease risk (OR = 2.24). Fine mapping and functional analysis of CD6 gene in Spanish–Basque dataset

Table 1: Demographic and clinical details of MS patients and healthy controls

	Case (*n*=300)	Control (*n*=720)
Age (mean±SD)	34.59±10.85	34.43±7.79
Male	99	343
Female	201	377
RRMS	243	-
PPMS	27	-
SPMS	30	-

SD = Standard deviation

Table 2: List of non-MHC SNPs in MS

Gene	SNP	Chromosome
CLEC 16A	rs6498169	16
IL2RA	rs2104286	10
CD6	rs17824933	11
RGS1	rs2760524	1
TYK2	rs34536443	19
TMEM39A	rs1132200	3
CD58	rs2300747	1
MPHOSPH 9	rs1790100	12
KIF21B	rs12122721	1
IL12A	rs4680534	3

SNPs = Single nucleotide polymorphisms, MS = Multiple sclerosis, MHC = Major histocompatibility complex

Table 3: SNP Frequency

		Results in indian population					Results in white population			
Gene	**SNP**	**Risk Allele**	**RAF case % (*n*=300)**	**RAF control % (*n*=720)**	***P* value**	**OR (95%CI)**	**RAF case %**	**RAF control %**	**OR**	**Ref**
CLEC16A	rs6498169	G	27.4	24.1	0.13	1.18 (0.95-1.47)	35	-	1.26 (1.15-1.38)	[6]
IL2RA	rs2104286	T	84.2	80.5	0.04	1.3 (1.006-1.67)	75	72	1.18 (1.12-1.24)	[6]
CD6	rs17824933	G	8.3	3.9	0.000042	2.24 (1.51-3.33)	25	19	1.18 (1.07-1.29)	[11]
RGS1	rs2760524	G	93.7	93.3	0.69	1.08 (0.73-1.60)	84	81	1.15 (1.10-1.22)	[6]
TYK2	rs34536443	G	99.3	99	0.49	1.47 (0.48-4.48)	96.6	95.3	1.39 (1.20-1.60)	[9]
TMEM39A	rs1132200	C	89.2	85.4	0.023	1.41 (1.05-1.91)	86	-	1.24 (1.10-1.32)	[27]
CD58	rs2300747	A	71.2	67.2	0.079	1.21 (0.98-1.49)	88	87	1.22 (1.10-1.34)	[8]
MPHOSP9	rs1790100	G	24.9	23.4	0.48	1.08 (0.87-1.35)	22	20	1.10 (1.00-1.22)	[11]
KIF21B	rs12122721	G	84.1	83.6	0.76	1.04 (0.80-1.35)	72	-	1.19 (1.07-1.32)	[27]
IL12A	rs4680534	C	15.2	15.3	0.96	0.99 (0.76-1.29)	36	26	1.12 (1.02-1.22)	[11]

SNP = Single nucleotide polymorphism, OR = Odds ratio, CI = Confidence interval, RAF = Risk allele frequency

revealed association of rs17824933 and rs11230559 with MS, both of which are strong in LD with each other ($r^2 > 0.8$). This data reinforce a genetic role for CD6 in susceptibility to MS.[26]

Very little has been known about TMEM39A (transmembrane protein 39A). The associated SNP (rs1132200) within this gene causes a nonsynonymous amino acid change (alanine-threonine) at position 487 in the protein. Genome-wide association study on two independent data set identifies TMEM39A as susceptibility loci (rs1132200, $P = 3.09 \times 10^{-8}$ OR = 1.24) for MS.[27] In a recent study on replication of TMEM39A (rs1132200) in 2863 Spanish MS patients and 2930 controls identified this gene as susceptibility gene for MS ($p_{M-H} = 0.001$, OR_{M-H} [95% CI] =0.84 [0.75–0.93]).[28] Our data showed nominal association for rs1132200 ($P = 0.023$, OR = 1.41, CI = 1.05–1.91). In this study, there was also a nominal association for IL2RA gene associated variant rs2104286 ($P = 0.04$, OR = 1.3, CI = 1.006–1.67). Fine mapping of IL2RA showed that rs2104286 was the most significant SNP in IL2RA region.[7] This gene associated SNP displayed significant associations with MS in a cohort of 1134 patients and 1265 controls from Australia ($P = 0.033$; OR = 0.86; 95% CI = 0.75–0.99),[29] and case-control, family collections from Europe population ($P = 6.27 \times 10^{-7}$ OR of 0.85, 95% CI = 0.79–0.92).[30] Meta-analysis on the two SNPs: rs2104286 and rs12722489 of IL2RA gene showed a positive association between IL2RA gene rs2104286 and MS.[31]

Though we failed to find a significant association in the majority of SNP's that were typed, frequency of the latter was more in MS patients as compared to healthy controls. In our previously published study of limited power, CD6 gene associated SNP had shown a trend of association.[16] Our recently published replication study on 110 European risk variants provides evidence that many if not all the MS risk variants identified in populations of European ancestry are likely to also be risk variants in the South Asian population. This study hypothesized that B cells and certain immunoglobulins might play an important role in the pathogenesis of MS in South Asian and other Asian populations in comparison to European MS patients.[17] Our data illustrate the similarity in risk association between Indian and European populations for MS.

Acknowledgment

This study was funded by Department of Science and Technology, Government of India (SR/SO/HS/127/2010).

Financial support and sponsorship

This study was funded by Department of Science and Technology, Government of India (SR/SO/HS/127/2010).

Conflicts of interest

There are no conflicts of interest.

References

1. Simon KC, Munger KL, Ascherio A. XVI European Charcot Foundation Lecture: Nutrition and environment: Can MS be prevented? J Neurol Sci 2011;311:1-8.
2. Sawcer S, Franklin RJ, Ban M. Multiple sclerosis genetics. Lancet Neurol 2014;13:700-9.
3. Sadovnick AD, Ebers GC, Dyment DA, Risch NJ. Evidence for genetic basis of multiple sclerosis. The Canadian Collaborative Study Group. Lancet 1996;347:1728-30.
4. Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, *et al.* Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. Nat Genet 2007;39:1083-91.
5. Matesanz F, Caro-Maldonado A, Fedetz M, Fernández O, Milne RL, Guerrero M, *et al.* IL2RA/CD25 polymorphisms contribute to multiple sclerosis susceptibility. J Neurol 2007;254:682-4.
6. International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, *et al.* Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 2007;357:851-62.
7. International Multiple Sclerosis Genetics Consortium (IMSGC). Refining genetic associations in multiple sclerosis. Lancet Neurol 2008;7:567-9.
8. De Jager PL, Baecher-Allan C, Maier LM, Arthur AT, Ottoboni L, Barcellos L, *et al.* The role of the CD58 locus in multiple sclerosis. Proc Natl Acad Sci U S A 2009;106:5264-9.
9. Ban M, Goris A, Lorentzen AR, Baker A, Mihalova T, Ingram G, *et al.* Replication analysis identifies TYK2 as a multiple sclerosis susceptibility factor. Eur J Hum Genet 2009;17:1309-13.
10. Hoppenbrouwers IA, Aulchenko YS, Janssens AC, Ramagopalan SV, Broer L, Kayser M, *et al.* Replication of CD58 and CLEC16A as genome-wide significant risk genes for multiple sclerosis. J Hum Genet 2009;54:676-80.
11. De Jager PL, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT, *et al.* Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet 2009;41:776-82.
12. Mero IL, Lorentzen AR, Ban M, Smestad C, Celius EG, Aarseth JH, *et al.* A rare variant of the TYK2 gene is confirmed to be associated with multiple sclerosis. Eur J Hum Genet 2010;18:502-4.

13. International Multiple Sclerosis Genetics Consortium; Well Come Trust Case Control Consortium, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, *et al.* Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476:214-9.
14. International Multiple Sclerosis Genetics Consortium (IMSGC), Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kemppinen A, *et al.* Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet 2013;45:1353-60.
15. Pandit L, Malli C, Singhal B, Wason J, Malik O, Sawcer S, *et al.* HLA associations in South Asian multiple sclerosis. Mult Scler 2016;22:19-24.
16. Pandit L, Ban M, Sawcer S, Singhal B, Nair S, Radhakrishnan K, *et al.* Evaluation of the established non-MHC multiple sclerosis loci in an Indian population. Mult Scler 2011;17:139-43.
17. Pandit L, Ban M, Beecham AH, McCauley JL, Sawcer S, D'Cunha A, *et al.* European multiple sclerosis risk variants in the south Asian population. Mult Scler 2016. pii: 1352458515624270.
18. Pandit L, Shetty R, Misri Z, Bhat S, Amin H, Pai V, *et al.* Optic neuritis: Experience from a south Indian demyelinating disease registry. Neurol India 2012;60:470-5.
19. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, *et al*. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292-302.
20. Reich D, Thangaraj K, Patterson N, Price AL, Singh L. Reconstructing Indian population history. Nature 2009;461:489-94.
21. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al.* PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
22. Isobe N, Gourraud PA, Harbo HF, Caillier SJ, Santaniello A, Khankhanian P, *et al.* Genetic risk variants in African Americans with multiple sclerosis. Neurology 2013;81:219-27.
23. Isobe N, Madireddy L, Khankhanian P, Matsushita T, Caillier SJ, Moré JM, *et al.* An ImmunoChip study of multiple sclerosis risk in African Americans. Brain 2015;138:1518-30.
24. Swaminathan B, Matesanz F, Cavanillas ML, Alloza I, Otaegui D, Olascoaga J, *et al.* Validation of the CD6 and TNFRSF1A loci as risk factors for multiple sclerosis in Spain. J Neuroimmunol 2010;223:100-3.
25. International Multiple Sclerosis Genetics Consortium. The genetic association of variants in CD6, TNFRSF1A and IRF8 to multiple sclerosis: A multicenter case-control study. PLoS One 2011;6:e18813.
26. Swaminathan B, Cuapio A, Alloza I, Matesanz F, Alcina A, García-Barcina M, *et al.* Fine mapping and functional analysis of the multiple sclerosis risk gene CD6. PLoS One 2013;8:e62376.
27. International Multiple Sclerosis Genetics Consortium (IMSGC). Comprehensive follow-up of the first genome-wide association study of multiple sclerosis identifies KIF21B and TMEM39A as susceptibility loci. Hum Mol Genet 2010;19:953-62.
28. Varadé J, Comabella M, Ortiz MA, Arroyo R, Fernández O, Pinto-Medel MJ, *et al.* Replication study of 10 genes showing evidence for association with multiple sclerosis: Validation of TMEM39A, IL12B and CBLB [correction of CLBL] genes. Mult Scler 2012;18:959-65.
29. Rubio JP, Stankovich J, Field J, Tubridy N, Marriott M, Chapman C, *et al.* Replication of KIAA0350, IL2RA, RPL5 and CD58 as multiple sclerosis susceptibility genes in Australians. Genes Immun 2008;9:624-30.
30. Maier LM, Lowe CE, Cooper J, Downes K, Anderson DE, Severson C, *et al.* IL2RA genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. PLoS Genet 2009;5:e1000322.
31. Wang LM, Zhang DM, Xu YM, Sun SL. Interleukin 2 receptor a gene polymorphism and risk of multiple sclerosis: A meta-analysis. J Int Med Res 2011;39:1625-35.