Genetic variants in *IL2RA* and *IL7R* affect multiple sclerosis disease risk and progression

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Abstract

Multiple sclerosis (MS) is a common demyelinating neurodegenerative disease with a strong genetic component. Previous studies have associated genetic variants in *IL2RA* and *IL7R* in the pathophysiology of the disease. In this study we describe the association between *IL2RA* (rs2104286) and *IL7R* (rs6897932) in the Canadian population. Genotyping 1,978 MS patients and 830 controls failed to identify any significant association between these variants and disease risk. However, stratified analysis for family history of disease, and disease course identified a trend towards association for *IL2RA* in patients without a family history ($p = 0.05$; odds ratio = 0.77), and a significant association between *IL7R* and patients who developed progressive MS (PrMS) ($p = 0.002$; odds ratio = 0.73).

Although not statistically significant, the effect of *IL2RA* (rs2104286) in patients without a family history of MS indicates that the genetic components for familial and sporadic disease are perhaps distinct. This data suggests the onset of sporadic disease is likely determined by a large number of variants of small effect, whereas MS in patients with a family history of disease is caused by a few deleterious variants. In addition, the significant association between PrMS and rs6897932 indicates that *IL7R* may not be disease-causing but a determinant of disease course. Further characterization of the effect of *IL2RA* and *IL7R* genetic variants in defined MS subtypes is warranted to evaluate the effect of these genes on specific clinical outcomes and to further elucidate the mechanisms of disease onset and progression.

Keywords

Multiple sclerosis; progressive disease; *IL2RA*; *IL7R*; association

INTRODUCTION

Multiple sclerosis (MS) is one of the most prevalent diseases of the central nervous system, affecting over two million people worldwide.[1] MS is a chronic inflammatory disease

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

characterized by myelin loss, varying degrees of axonal pathology, and progressive neurological dysfunction;[2,3] the symptoms vary widely and may affect vision, hearing, cognition, balance, and movement.

Genetic susceptibility factors resulting in an increased predisposition to developing MS are known to exist, with the human leucocyte antigen (HLA)-DRB1 region providing the strongest risk; however, additional risk factors have been described.[4,5] Outside of the HLA region, interleukin 2 receptor alpha (*IL2RA*) and interleukin 7 receptor (*IL7R*) are two of the genes more consistently found associated with risk of MS.[6,7] A meta-analysis of *IL2RA* rs2104286 and *IL7R* rs6897932 have confirmed these associations in the Caucasian population,[8,9] including Canadian patients.[10] Interestingly, one study found *IL7R* rs6897932 to be exclusively associated with patients presenting primary progressive MS (PPMS) and secondary progressive MS (SPMS), suggesting *IL7R* may have an effect on the development of a progressive disease course.[11]

To further elucidate the role of *IL2RA* and *IL7R* in MS pathogenesis and disease course, we genotyped rs2104286 and rs6897932 in a large Canadian case-control series consisting of 1,978 MS patients and 830 controls, and sequenced the entire coding region for each gene in 95 MS patients.

MATERIALS and METHODS

Genetic analysis of *IL2RA* and *IL7R* were performed in MS patients and unrelated healthy controls collected through the longitudinal Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) between 1997 and 2008.[12] The ethical review boards at the University of British Columbia approved the study, and all participants provided informed consent. All patients were diagnosed with MS according to Poser criteria prior to 2001,[13] or McDonald criteria thereafter.[14,15] A total of 1,978 MS patients and 830 controls were characterized in this study. The mean age at blood collection was 46.7 years ($SD \pm 11.7$) for MS patients and 66.9 years ($SD \pm 9.7$) for controls, with a male to female ratio of 1:2.77 and 1:0.97 respectively. The mean age at MS onset was 30.9 years ($SD \pm 9.7$), with a median Expanded Disability Status Scale (EDSS) score of 3.5 and an average of 4.0 ($SD \pm 2.6$).[16] Detailed clinical course was available for 1,632 patients (82.5%); the majority presented a clinical course consistent with relapsing remitting MS (RRMS) (54.6%), and the remainder presented PPMS (25.0%) or SPMS (20.4%). A positive family history of disease, defined as a having at least one first or second degree relative also diagnosed with MS was present in 82.5% of cases. The higher than usual frequency of patients with a family history of disease, and as a consequence the increased number of patients with PPMS, is the result of our research protocol which favored the collection of familial probands.

IL2RA rs2104286 and *IL7R* rs6897932 were genotyped on an ABI 7900 using TaqMan probes and analyzed with SDS 2.4 software. Genotypic associations were examined by Chi-square test. Bonferroni correction for multiple testing was applied, and p-values < 0.005 are considered significant after adjustment for 10 independent tests (two variants in five disease groups). All SNPs were consistent with Hardy-Weinberg equilibrium (p-value > 0.05).

Primer pairs were designed for specific amplicons containing all coding exons and exon-intron boundaries for *IL2RA* and *IL7R* (sequences available on request). PCR products were generated using standard protocols. Magnetic-bead based PCR purification was achieved with Agencourt technology. Sequencing products were analyzed on an ABI 3730xl capillary array and SeqScape software as previously described.[17]

RESULTS

Power analysis

A priori power analysis assuming a disease prevalence of 0.001,[1] with a minor allele frequency of 0.25, as observed in Caucasian populations, and the previously described relative risk of 1.25,[8,9] indicated that our series (n=2,808) has a 93% probability of identifying nominally significant differences in genotype frequencies between MS patients and controls. Analysis of MS subgroups, assuming the same parameters, indicated a statistical power of 91% for familial MS, 59% for sporadic MS, 83% for RRMS and 79% for progressive MS (PrMS; PPMS and SPMS).

Interleukin 2 receptor alpha

Association analysis of *IL2RA* rs2104286 in our Canadian MS series consisting of 1,978 patients and 830 controls showed no significant differences in genotype or allelic frequencies between cases and controls (Table 1). Although significant associations were not identified, carriers of the rs2104286 G allele presented a protective odds ratio (OR) of 0.87 (95% CI = 0.74–1.03) (Figure 1), similar to those previously reported.[8,7] To further characterize the role of *IL2RA* in MS, we performed stratified association analyses according to family history of disease, as well as disease progression. Differences in rs2104286 genotypic or allelic frequencies were not observed between healthy controls and patients with positive family history of disease or progressive disease (Table 1). However, a trend toward association for *IL2RA* genotypes was identified in MS patients without a family history of disease (p-value = 0.05, OR = 0.77; 95% CI = 0.60–1.00) and to a lesser extent in patients with a disease course consistent with RRMS (p-value = 0.05, OR = 0.86; 95% CI = 0.71–1.05) (Figure 1).

In an attempt to identify the biologically relevant variants affecting MS risk, we sequenced the entire coding region of *IL2RA* in 95 randomly selected patients without family history of disease. Sequencing analysis identified two silent and two missense variants (Table 2). All four variants had been previously reported, and did not present an allelic frequency sufficiently distinct from the general population to account for the difference in disease risk observed in this group.

Interleukin 7 receptor

Genotyping of rs6897932 in MS cases and controls did not result in a statistically significant association between genotypic or allelic frequencies and risk of MS (p-value = 0.07) (Table 1), although a trend towards association, with a protective OR of 0.87 (95% CI = 0.74–1.02) for TT/CT vs CC genotypes was observed (Figure 1). Analysis in MS patients stratified by family history of disease did not result in a significant association, with ORs for familial and

sporadic patients similar to those obtained for the entire cohort. Similarly, stratified analysis for disease course did not reveal a significant association between *IL7R* and patients presenting RRMS; however a statistically significant association was observed between patients diagnosed with PrMS and rs6897932 genotype (p-value = 0.002) (Table 1). This association appears to be primarily driven by those patients suffering from PPMS (p-value = 0.001, OR = 0.68; 95% CI = 0.53–0.86) as those with SPMS are not significantly different from healthy controls (p-value = 0.06, OR = 0.80; 95% CI = 0.62–1.03).

The associated rs6897932 C to T transition results in a threonine to isoleucine substitution at position 244 (p.T244I). In vitro studies have shown increased skipping of *IL7R* exon 6 when the C allele is present, thus potentially altering the ratio between membrane-bound and soluble protein.[6] To investigate whether other biologically relevant variants in *IL7R* could account for the observed associations we sequenced its entire coding region in 95 randomly selected MS probands. This analysis identified two silent and seven missense variants, including p.T244I (Table 2). The minor allele frequency (MAF) for the identified variants was relatively similar to those previously reported, with the largest difference in allelic frequency being observed for p.T244I and p.I356V with an 8% decrease and 9% increase respectively.

DISCUSSION

The implementation of ever larger genome wide association studies of MS has nominated a myriad of variants with a potential role in disease susceptibility; amongst those, variants located within the HLA system appear to account for the largest effect on disease risk.[4] Two of the non-HLA variants which have been substantially replicated in independent studies, albeit not consistently, are the rs2104286 in *IL2RA* and rs6897932 in *IL7R*. [8,9] These two genes are plausible biological candidates for MS since the proteins they encode, interleukin 2 receptor alpha (*IL2Rα*, also known as CD25) and interleukin 7 receptor alpha (*IL7Rα*, also known as CD127), play a fundamental role in lymphocyte development and the modulation of T cell effector function.[18,19]

The goal of this study is to further define the role of *IL2RA* rs2104286 and *IL7R* rs6897932 in the susceptibility to MS. To this end, we characterized these two variants in a case-control series of MS patients from Canada. This cohort is noticeably distinct from those described in most association studies of *IL2RA* and *IL7R* with MS,[8,9] as it contains a higher proportion of patients with a family history of disease (82.5%), as well as a higher number of patients presenting PPMS (25%). Interestingly neither variant resulted in a significant association with disease in the entire cohort (Table 1) despite having a 93% power of detection. Although not significantly associated, the minor allele for both variants presented a clear trend towards a protective effect as previously described (Figure 1).

Stratified analysis for *IL2RA* indicates that the trend towards association is driven by the patients without a family history of disease, thus suggesting that genetic components for familial and sporadic disease may be somewhat distinct. This is not unexpected if we assume that sporadic disease is caused by the accumulation of genetic and environmental variants of small individual effect, whereas the onset of disease for familial forms is determined by only

a few variants of stronger effect.[20] In this scenario, the small difference in disease risk resulting from genetic variants in *IL2RA* (OR=0.77) is likely to have a more profound outcome on sporadic patients, as the accumulation of variants of small effects is necessary to reach a minimum risk threshold for the onset of MS. In contrast, the influence of variants of small effect in patients with a family history of disease will likely be minor on the overall disease risk, if a few variants of strong effect are already present. Sequencing of *IL2RA* in MS patients without family history of disease did not identify coding variants that could account for the observed association, thus suggesting that the effect on MS risk is unlikely to be driven by amino acid substitutions.

Similarly, the association of *IL7R* with MS resulted in a non-significant trend in the entire cohort; however, analysis of clinical subtypes identified a strong association between rs6897932 and patients presenting PrMS (Table 1). Our study replicates previous reports of association between *IL7R* variants with PrMS, emphasizing the existence of specific genetic components implicated in the development of progressive disease.[11,21] Sequencing of *IL7R* in MS patients identified two missense variants (rs6897932, p.T244I and rs3194051, p.I356V) with an allelic frequency difference greater than 8% from those expected in the general population (Table 2). The p.T244I variant, genotyped in this study, has been shown to affect alternative splicing of *IL7R* exon 6 and alter the amounts of soluble and membrane-bound protein, thus suggesting a possible mechanism for disease.[6,21] In contrast, no functional outcome has been described for p.I356V, and association studies for this variant (rs3194051) have provided conflicting results, with some studies reporting significant association with risk of MS,[22–24] that others could not replicate.[6,25]

In summary, our study has confirmed the association between genetic variants in *IL2RA* and *IL7R* in MS, and further defined this association to specific disease subtypes. Our data suggests that the major genetic components in sporadic and familial MS are distinct, with common variants of small effect being critical for sporadic disease, and a small number of variants of greater effect for familial MS. In addition we have replicated the association of *IL7R* rs6897932 in patients with PrMS, reinforcing the notion that the absence of remission seen in PPMS patients may be the result of a decrease in soluble IL7Rα in regulatory T cells,[21,26] thus providing a mechanistic target for therapeutic intervention.

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References

1. Wade BJ. Spatial analysis of global prevalence of multiple sclerosis suggests need for an updated prevalence scale. *Mult Scler Int.* 2014; 2014:124578.doi: 10.1155/2014/124578 [PubMed: 24693432]
2. Ebers GC. Environmental factors and multiple sclerosis. *Lancet Neurol.* 2008; 7(3):268–277. S1474-4422(08)70042-5. [PubMed: 18275928]

3. Noseworthy JH. Progress in determining the causes and treatment of multiple sclerosis. *Nature*. 1999; 399(6738 Suppl):A40–47. [PubMed: 10392579]
4. Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011; 476(7359):214–219. DOI: 10.1038/nature10251 [PubMed: 21833088]
5. Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kempainen A, Cotsapas C, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet*. 2013; 45(11):1353–1360. DOI: 10.1038/ng.2770 [PubMed: 24076602]
6. 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(9):1083–1091. DOI: 10.1038/ng2103 [PubMed: 17660817]
7. Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med*. 2007; 357(9):851–862. DOI: 10.1056/NEJMoa073493 [PubMed: 17660530]
8. Wang LM, Zhang DM, Xu YM, Sun SL. Interleukin 2 receptor alpha gene polymorphism and risk of multiple sclerosis: a meta-analysis. *J Int Med Res*. 2011; 39(5):1625–1635. [PubMed: 22117963]
9. Zhang R, Duan L, Jiang Y, Zhang X, Sun P, Li J, Zhang M, Tang G, Wang X, Li X. Association between the IL7R T244I polymorphism and multiple sclerosis: a meta-analysis. *Mol Biol Rep*. 2011; 38(8):5079–5084. DOI: 10.1007/s11033-010-0654-5 [PubMed: 21161391]
10. Hoppenbrouwers IA, Aulchenko YS, Janssens AC, Ramagopalan SV, Broer L, Kayser M, Ebers GC, Oostra BA, van Duijn CM, Hintzen RQ. Replication of CD58 and CLEC16A as genome-wide significant risk genes for multiple sclerosis. *J Hum Genet*. 2009; 54(11):676–680. DOI: 10.1038/jhg.2009.96 [PubMed: 19834503]
11. Akkad DA, Hoffjan S, Petrasch-Parwez E, Beygo J, Gold R, Epplen JT. Variation in the IL7RA and IL2RA genes in German multiple sclerosis patients. *J Autoimmun*. 2009; 32(2):110–115. DOI: 10.1016/j.jaut.2009.01.002 [PubMed: 19231135]
12. Sadovnick AD, Risch NJ, Ebers GC. Canadian collaborative project on genetic susceptibility to MS, phase 2: rationale and method. Canadian Collaborative Study Group. *Can J Neurol Sci*. 1998; 25(3):216–221. [PubMed: 9706723]
13. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol*. 1983; 13(3):227–231. DOI: 10.1002/ana.410130302 [PubMed: 6847134]
14. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol*. 2001; 50(1):121–127. [PubMed: 11456302]
15. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. *Ann Neurol*. 2005; 58(6):840–846. DOI: 10.1002/ana.20703 [PubMed: 16283615]
16. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33(11):1444–1452. [PubMed: 6685237]
17. Trinh J, Vilarino-Guell C, Donald A, Shah B, Yu I, Szu-Tu C, Aasly JO, Wu RM, Hentati F, Rajput AH, Rajput A, Farrer MJ. STX6 rs1411478 is not associated with increased risk of Parkinson's disease. *Parkinsonism Relat Disord*. 2013; 19(5):563–565. DOI: 10.1016/j.parkreldis.2013.01.019 [PubMed: 23415606]
18. Jiang Q, Li WQ, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, Durum SK. Cell biology of IL-7, a key lymphotrophin. *Cytokine Growth Factor Rev*. 2005; 16(4–5):513–533. S1359-6101(05)00062-6. [PubMed: 15996891]
19. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. *Nat Rev Immunol*. 2004; 4(9):665–674. DOI: 10.1038/nri1435 [PubMed: 15343366]
20. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009; 461(7265):747–753. DOI: 10.1038/nature08494 [PubMed: 19812666]

21. Booth DR, Arthur AT, Teutsch SM, Bye C, Rubio J, Armati PJ, Pollard JD, Heard RN, Stewart GJ. Gene expression and genotyping studies implicate the interleukin 7 receptor in the pathogenesis of primary progressive multiple sclerosis. *J Mol Med (Berl)*. 2005; 83(10):822–830. DOI: 10.1007/s00109-005-0684-y [PubMed: 16075257]
22. Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M, Oturai A, Ryder LP, Saarela J, Harbo HF, Celius EG, Salter H, Olsson T, Hillert J. Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. *Nat Genet*. 2007; 39(9):1108–1113. ng2106. [PubMed: 17660816]
23. Zhang Z, Duvefelt K, Svensson F, Masterman T, Jonasdottir G, Salter H, Emahazion T, Hellgren D, Falk G, Olsson T, Hillert J, Anvret M. Two genes encoding immune-regulatory molecules (LAG3 and IL7R) confer susceptibility to multiple sclerosis. *Genes Immun*. 2005; 6(2):145–152. 6364171. [PubMed: 15674389]
24. Qiu W, Pham K, James I, Nolan D, Castley A, Christiansen FT, Czarniak P, Luo Y, Wu J, Garlepp M, Wilton S, Carroll WM, Mastaglia FL, Kermode AG. The influence of non-HLA gene polymorphisms and interactions on disease risk in a Western Australian multiple sclerosis cohort. *J Neuroimmunol*. 2013; 261(1–2):92–97. DOI: 10.1016/j.jneuroim.2013.04.022 [PubMed: 23726763]
25. O'Doherty C, Kantarci O, Vandenbroeck K. IL7RA polymorphisms and susceptibility to multiple sclerosis. *N Engl J Med*. 2008; 358(7):753–754. DOI: 10.1056/NEJMc0707553 [PubMed: 18272905]
26. McKay FC, Swain LI, Schibeci SD, Rubio JP, Kilpatrick TJ, Heard RN, Stewart GJ, Booth DR. CD127 immunophenotyping suggests altered CD4+ T cell regulation in primary progressive multiple sclerosis. *J Autoimmun*. 2008; 31(1):52–58. DOI: 10.1016/j.jaut.2008.02.003 [PubMed: 18406576]

Figure 1.

Forest plot of the odds ratio (OR) and 95% confidence intervals (CI) for *IL2RA* rs2104286 (GG/AG vs. AA) and *IL7R* rs6897932 (TT/CT vs. CC). The size of each black box represents the proportional sample size of each stratified analysis, with the horizontal line indicating its corresponding 95% CI.

Table 1

Genotype frequencies and p-values for *IL2RA* rs2104286 and *IL7R* rs6897932. Statistically significant associations are indicated in bold.

Group	<i>IL2RA</i> rs2104286 n(%)			p-value	
	AA	AG	GG	Genotype	Allele
Controls	463 (55.8)	318 (38.3)	49 (5.9)		
All patients	1168 (59.0)	700 (35.4)	110 (5.6)	0.109	0.147
Familial	953 (58.4)	586 (35.9)	92 (5.6)	0.208	0.259
Sporadic	215 (62.0)	114 (32.9)	18 (5.2)	0.050	0.074
Relapsing	529 (59.4)	302 (33.9)	60 (6.7)	0.053	0.346
Progressive	422 (57.0)	285 (38.5)	34 (4.6)	0.240	0.419

Group	<i>IL7R</i> rs6897932 n(%)			p-value	
	CC	CT	TT	Genotype	Allele
Controls	453 (54.6)	324 (39.0)	53 (6.4)		
All patients	1149 (58.1)	721 (36.5)	108 (5.5)	0.074	0.077
Familial	943 (57.8)	596 (36.5)	92 (5.6)	0.117	0.125
Sporadic	206 (59.4)	125 (36.0)	16 (4.6)	0.089	0.093
Relapsing	501 (56.2)	344 (38.6)	46 (5.2)	0.246	0.332
Progressive	461 (62.2)	238 (32.1)	42 (5.7)	0.002	0.006

Table 2

Coding variants identified by sequencing *IL2RA* (NM_000417.2) and *IL7R* (NM_002185.3) in MS patients. Minor allele frequencies (MAF) for this subset of patients and samples of European descent from the NHLBI GO Exome Sequencing Project (ESP) database are provided.

<i>IL2RA</i>				
dbSNP	Nucleotide	Amino acid	MAF	ESP
rs2228150	G>A	P28P	0.037	0.027
rs201188114	G>A	V153I	0.005	0
rs147791171	G>A	V167I	0.005	0
rs2228149	C>T	H172H	0.047	0.036

<i>IL7R</i>				
dbSNP	Nucleotide	Amino acid	MAF	ESP
rs1494558	C>T	I66T	0.285	0.326
rs1494555	A>G	V138I	0.280	0.320
rs2228141	C>T	H165H	0.170	0.142
rs6897932	C>T	T244I	0.183	0.267
rs41270321	A>C	N348T	0.021	0.017
rs3194051	A>G	I356V	0.356	0.264
rs201671392	T>C	S366P	0.005	0
rs2229232	C>T	T414M	0.005	0.005
rs201755068	A>G	Q422Q	0.005	0