SHORT REPORT

MYO9B polymorphisms in multiple sclerosis

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Single-nucleotide polymorphisms (SNPs) in the 3' region of myosin IXB (*MYO9B*) gene have recently been reported to associate with different inflammatory or autoimmune diseases. We monitored for the association of *MYO9B* variants to multiple sclerosis (MS) in four Northern European populations. First, 18 SNPs including 6 SNPs with previous evidence for association to immune disorders, were tested in 730 Finnish MS families, but no linkage or family-based association was observed. To ensure the power to detect variants with a modest effect size, we further analyzed 10 variants in 899 Finnish cases and 1325 controls, and in a total of 1521 cases and 1476 controls from Denmark, Norway and Sweden, but found no association. Our results thereby do not support a major function of the tested *MYO9B* variants in MS.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, which leads to demyelination and neurodegeneration. Although the exact etiology of MS is unknown, it is considered a putative autoimmune disease (AID) caused by a combination of multiple genetic and environmental factors.¹ Like many AIDs, MS has consistently shown strong linkage and association to the human leukocyte antigen (HLA) locus.

Figure 1 Haploblock structure of *MYO9B* based on HapMap CEPH genotype data. The coloring reflects level of linkage disequilibrium between single-nucleotide polymorphisms (SNPs) in HapMap CEPH data, darker shades representing higher pairwise r^2 . The 10 SNPs genotyped in all samples are indicated with black arrows. SNPs genotyped in Finnish MS families only are indicated with gray arrows. SNPs previously showing association to other disorders are marked with asterisks.

In addition to the HLA, also other loci such as *PTPN22* and *CTLA4*, have shown linkage or association with more than one AID, indicating that AIDs may share some common genetic background.² More recently, allelic variants in the gene encoding for myosin IXB (*MYO9B*) have been reported to associate to different AIDs or inflammatory disorders (ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus and type 1 diabetes) following the initial report by Monsuur *et al*³ in which association to celiac disease (CD) was found with six *MYO9B* 3' region single-nucleotide polymorphisms (SNPs) in the Dutch population.^{3–8} To further investigate the relevance of *MYO9B* in different immune disorders, we have tested the association of its genetic variants to MS in four Northern European populations.

Materials and methods

Subjects and genotyping

The Finnish MS family sample consisted of 730 families (340 full trios). Nineteen families were informative for linkage containing 2–4 affected individuals and available healthy family members. In the other families, one affected and at least one healthy parent or sibling were genotyped. The family cases were also included in a case–control analysis with 169 additional Finnish MS patients, 1325 unrelated Finnish population controls and 1521 MS cases and 1476 population controls from Denmark, Norway and Sweden. All cases had been diagnosed according to Poser's criteria and informed consent was obtained from all subjects. The study was approved by the ethics committees of all institutions involved.

Table 1 Results from family-based association analysis

SNP	Alleles (major: minor)	Family-based analysis	
		TDT P-value	Gamete competition P-value
rs3745349	C:T	0.55	0.60
rs7246865	G:A	0.61	0.89
rs8099879	C:A	0.63	0.19
rs7254359	A:G	0.91	0.60
rs10423232	T:C	0.70	0.11
rs17533868	C:T	0.73	0.22
rs17533945	T:C	0.74	0.84
rs11673417	C:G	0.41	0.44
rs12986130	C:A	0.26	0.18
rs2279006	C:T	0.58	0.03
rs3826689	G:T	0.11	0.29
rs962917	G:A	0.13	0.48
rs1545620	T:G	0.16	0.40
rs1457092	C:A	0.22	0.66
rs2279003	C:T	0.51	0.65
rs8107108	C:T	0.47	0.52
rs2305765	C:T	0.12	0.54
rs2305764	G:A	0.30	0.71

Abbreviations: SNP, single-nucleotide polymorphism; TDT, transmission disequilibrium test.

Eighteen tagging SNPs selected to represent all major haploblocks in *MYO9B* were genotyped in 730 Finnish MS families in two multiplexes (Figure 1). All genotyping was conducted with Sequenom iPLEX platform (Sequenom Inc., San Diego, CA, USA, detailed protocols and primer sequences available on request). One of the multiplex assays (10 SNPs) was then genotyped in cases and controls. These 18 and 10 SNPs, respectively, capture 56 and 48% of the variation in *MYO9B* in HapMap CEPH samples with pairwise $r^2=0.7$ (phase II data).⁹ Overall genotyping success rate per SNP was 97–100% in all populations.

Statistical analysis

Power calculations were performed with the Genetic Power Calculator.¹⁰ Haploview and PedCheck were used for data quality checks (Hardy–Weinberg equilibrium P -value ≥ 0.01 in healthy family founders, cases and controls in all populations, at most one Mendelian error/SNP).^{11,12} Family-based analyses were performed with Merlin (nonparametric linkage test using Kong and Cox LOD score),¹³ transmission disequilibrium test (TDT; Haploview) and Gamete competition test (in Mendel software package version 7.0), which uses families with missing genotype data in addition to full trios.^{14,15} Case–control association test was conducted as a two-tailed χ^2 -test in Haploview.

Results and discussion

We first genotyped 18 SNPs including 6 SNPs with previous association to other AIDs in 730 Finnish MS families. The power to detect association at rs2305764 ($P=0.05$) in TDT

Table 2 Results from case–control analysis

SNP	Alleles major: minor	Finland, n (cases) = 899, n (controls) = 1325		Denmark, n (cases) = 488, n (controls) = 468		Norway, n (cases) = 348, n (controls) = 369		MAF Sweden, n (cases) = 685, n (controls) = 639	
		MAF cases: controls	P-value	MAF cases: controls	P-value	MAF cases: controls	P-value	MAF cases: controls	CMH P-value
rs3745349	C:T	0.17:0.16	0.66	0.21:0.22	0.53	0.19:0.21	0.93	0.19:0.19	0.97
rs7246865	G:A	0.20:0.20	0.99	0.24:0.25	0.60	0.24:0.22	0.45	0.24:0.23	0.65
rs10423232	T:C	0.19:0.16	0.02	0.17:0.20	0.20	0.18:0.15	0.47	0.17:0.18	0.21
rs17533868	C:T	0.04:0.03	0.23	0.04:0.04	0.96	0.04:0.02	0.66	0.03:0.03	0.12
rs17533945	T:C	0.49:0.48	0.45	0.40:0.39	0.81	0.41:0.39	0.58	0.41:0.41	0.42
rs11673417	C:G	0.04:0.04	0.72	0.01:0.02	0.32	0.02:0.02	0.34	0.02:0.02	0.48
rs12986130	C:A	0.46:0.45	0.48	0.34:0.33	0.96	0.35:0.39	0.22	0.39:0.36	0.55
rs2279006	C:T	0.07:0.07	0.99	0.09:0.09	0.96	0.06:0.05	0.54	0.06:0.08	0.40
rs3826689	G:T	0.41:0.43	0.39	0.41:0.38	0.31	0.37:0.37	0.84	0.40:0.39	0.98
rs1457092	C:A	0.40:0.41	0.35	0.37:0.35	0.30	0.34:0.34	0.97	0.37:0.36	0.97

Abbreviations: CMH, Cochran–Mantel–Haenszel; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

was 0.96 assuming risk allele frequency of 35.7% and odds ratios 1.66 and 2.27 for heterozygotes and homozygotes, respectively, as reported by Monsuur *et al*³ for CD. We did not observe significant evidence of association in single SNP TDT or gamete competition tests (Table 1), or in haplotype TDT. Furthermore, no evidence of linkage was observed (Merlin nonparametric single point LOD scores 0.59–0.72).

We acknowledge, however, that with lower effect sizes our family sample may have been underpowered to detect association. We therefore genotyped a set of Northern European MS cases and controls for a single multiplex of 10 SNPs. This included rs1457092, which efficiently tags the CD risk haplotype identified by Monsuur *et al*,³ and has been among the most strongly and consistently associated SNPs in other studies. Concordantly with our results from the family-based analysis, we failed to see evidence for association in any single population or in combined analysis (Cochran–Mantel–Haenszel test; Table 2). Furthermore, no association was observed after stratifying the cases for gender or HLA-DRB1*15 (results not shown).

Although association of MYO9B has been reported to more than one AID or inflammatory disease, its role remains controversial because of failed replication efforts.^{16–21} In this study we tested the association of MYO9B variants to yet another inflammatory disorder, MS, but failed to find association. This is concordant with the lack of association with MYO9B in the recent international genomewide association study for MS,²² which, however, contained no SNPs in the previously associated 3' haplotype block. Considering the reasonable power of our sample, the previously AID-associated MYO9B variants are unlikely to be involved in MS.

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