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Fine mapping of the multiple sclerosis susceptibility locus on 5p14–p12

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Abstract

Linkage analyses have identified four major MS susceptibility loci in Finns. Here we have fine mapped the region on chromosome 5p in 28 Finnish MS families. Marker D5S416 provided the highest pairwise LOD score, and multipoint and haplotype analyses restrict the critical region to about 5.3 Mb on 5p15 between markers D5S1987 and D5S416. Ascertaining for HLA type and geographical origin indicated that families with and without the HLA DR15 risk haplotype, as well as families within and outside an internal high-risk region, contributed to the linkage to 5p, implying the general significance for this locus in Finnish MS families.

Keywords: Multiple sclerosis; Fine mapping; Linkage analysis

1. Introduction

Multiple sclerosis (MS) is a chronic neurological disorder characterized by multicentric inflammation, demyelination and axonal damage. The prevalence of MS is about 0.5–1 in 1000 in populations of Northern European origin, and the age of onset is between 20–40 years of age (Kurtzke, 1983). The most common clinical features include sensory loss, motor weakness, visual, bowel and bladder disturbances and cognitive deficits (Noseworthy et al., 2000).

The etiology of MS is complex, but twin-, adoption- and other epidemiological studies have all suggested a significant genetic component in MS (Dyment et al., 2004a). Several genome-wide scans have been performed in different populations using families with multiple affected individuals, revealing some linkage evidence for numerous putative MS loci (Ebers et al., 1996; Haines et al., 1996,

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Sawcer et al., 1996; Kuokkanen et al., 1997; Broadley et al., 2001; Coraddu et al., 2001; Akesson et al., 2002; Ban et al., 2002; Haines et al., 2002; Eraksoy et al., 2003a; Hensiek et al., 2003; Dyment et al., 2004b). Only the MHC region on chromosome 6p has consistently been replicated. However, linkage evidence for regions on chromosomes 5p and 17q emerged in several scans (Dyment et al., 2004a; Eraksoy et al., 2003a), and the region on 17q was singled out in a meta-analysis of nine genome scans (GAMES and the Transatlantic Multiple Sclerosis Genetics Cooperative, 2003).

Previous linkage analyses in Finnish MS families have indicated four main MS candidate loci: the MHC-region on chromosome 6p (Tienari et al., 1993, 1998), the MBP-locus on chromosome 18q (Tienari et al., 1992) and two relatively wide regions on chromosomes 5p12–p14 (Kuokkanen et al., 1996) and 17q22–q24 (Kuokkanen et al., 1997). The locus on chromosome 17 was further restricted to a 3.4 Mb region on 17q24 (Saarela et al., 2002). Interestingly, the loci on 5p and 17q are syntenic to the mouse experimental allergic encephalomyelitis (*Eae*) 2 (Sundvall et al., 1995) and rat *Eae* (Jagodic et al., 2001) loci, respectively.

By fine mapping the wide susceptibility locus on 5p and monitoring for haplotype sharing among affected individuals in Finnish MS families, we have here restricted the critical region to about 5.3 Mb on 5p15, providing the starting point for molecular analysis of regional candidate genes.

2. Materials and methods

2.1. MS families

Initially, the study material consisted of 26 multiplex MS families. These families are identical to those previously reported (Tienari et al., 1992; Kuokkanen et al., 1996, 1997; Saarela et al., 2002), with some modifications for families 1, 2, 5 and 9: For the original families 1, 2 and 5 (Kuokkanen et al., 1996, 1997), we observed that only affected individuals within certain branches of the pedigrees shared a haplotype, and these shared haplotypes originated from individuals married into the family. Thus, families 1, 2, and 5 were split into 1A–B, 2A–B and 5A–B, respectively, to reduce the apparent bilinearity. One member of family 9 was previously diagnosed with optic neuritis, but has later developed clinically definitive MS. Her cousin has also developed MS, and this patient and her father have been included in our study. Thus our principal study material was 28 multiplex families with 2–4 affected individuals per family (Table 1, sets 1a–b). In addition, extended study samples of 166 (Table 1, sets 2a–b) and 250 (Table 1, sets 3a–b) singleton cases with parents and/or unaffected sibs (Saarela et al., 2002) were included in our analyses. Overall, 22 multiplex families (Table 1, set 1a) and 134 singleton cases (Table 1, sets 2a–3a) originated from the Southern Ostrobothnia region, which is a regional sub-isolate in the southwestern part of Finland with prevalence of MS over twice as high as elsewhere in Finland (Wikström, 1975; Sumelahti et al., 2000, 2001). Diagnosis of MS was performed according to Posers criteria (Poser et al., 1983).

2.2. Genotyping of microsatellite markers

Genomic DNA was extracted from peripheral blood cells following standard protocols. We selected 26 microsatellite markers from public databases (GDB (www.gdb.edu), NCBI (www.ncbi.nlm.nih.org), Marshfield (research.marshfieldclinic.org/genetics/) and Whitehead/MIT (www-genome.wi.mit.edu/cgi-bin/contig/phys_map). IRD-700 or IRD-800 fluorescently labeled primers were from LI-COR Inc., VIC labeled primer sets were from Applied Biosystems, all other primers were from Operon or Gibco BRL. Forward LI-COR primers were 5′ tailed with “GTGTCTT” to facilitate genotyping of dinucleotides repeats, as recommended by LI-COR Inc. PCR was carried out as single reactions using AmpliTaq Gold DNA polymerase or Platinum Taq polymerase (both Applied Biosystems). [Specific PCR conditions can be obtained from author HMFRS by request.] PCR products were pooled and subjected to PAGE on a Global IR2 System (LI-COR Inc.), or run on an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). LI-COR gels were analyzed using the Saga1.0 software (University of Washington and LI-COR Inc.), ABI 3700 runs were analyzed using the

Table 1
Summary of the MS study sample

Dataset	#Families	#Affecteds	#Typed affecteds	Of the typed affecteds:			
				#Female	#Male	#DR15	#DRX
Multiplex set 1a (SO)	22	65	56	40	16	35	21
Multiplex set 1b	6	16	15	9	6	12	3
Singleton set 2a (SO)	37 (42*)	37 (42)	37 (42)	29 (30)	8 (12)	0	2
Singleton set 2b	124	124	124	80	44	56	40
Singleton set 3a (SO)	92	92	92	63	29	na	na
Singleton set 3b	158	158	158	113	45	na	na
Total	439 (444)	492 (497)	482 (487)	334 (335)	148 (152)	103	66

SO=from Southern Ostrobothnia; DR15=affected individuals that have the HLA DR15 type; DRX=affected individuals that do not have the HLA DR15 type (HLA data from Tienari et al., 1993; Laaksonen et al., 2002); na=not analysed; *=5 additional families were included in the SNP analyses.