A genome-scan in a single pedigree with a high prevalence of MS

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Abstract

Background: Multiple sclerosis (MS) is a disease widely believed to be autoimmune in nature. Genetic-epidemiological studies implicate susceptibility genes in pathogenesis, although non-MHC susceptibility linkages have been difficult to confirm. Insight into pathways intrinsic to other complex diseases has come from the genetic analysis of large, autosomal dominant kindreds. Here we present a genetic study of a large and unique kindred in which MS appears to follow an autosomal dominant pattern of inheritance with consistent penetrance in 4 generations.

Methods: Eighty-two individuals of this 370 member family were genotyped with 681 microsatellite markers spanning the genome with an average spacing of 5.3 cM. Results: Parametric linkage analysis was performed and no significant LOD score (LOD>3.3) was observed. For a rare dominant disease model with reduced penetrance, 99.6% of the genome was excluded at a lod score <-1 and 96% at a lod score <-2. The HLA-DRB1 candidate gene was also genotyped by allele-specific methods. In each instance where at least one parent was positive for HLA-DRB1*15, one or more HLA-DRB1*15 alleles were transmitted to the affected offspring (11/11). *HLA-DRB1*15* was transmitted equally from both the familial and the married-in parents and therefore this locus does not appear to be an autosomal dominant acting gene in this family but an important modifier of risk.

Conclusions: These results further stress the importance of the *HLA-DRB1*15* bearing haplotype in determining MS susceptibility. Furthermore this study highlights the complexity MS genetics; even in the presence of a single family seemingly segregating MS as an autosomal dominant trait.

Introduction

Multiple sclerosis (MS) is a complex disease of unknown etiology[1] although both environmental factors and susceptibility genes are thought to play a role in its pathogenesis. There is a marked familial aggregation as twenty percent of MS patients have another relative with MS and a first degree relative is 18-36 times more likely to have MS compared to the general population[2,3]. The concordance rate in identical twins is 25-30%, and the rate in fraternal twins is much less at approximately 5.4%, which may be truly higher than the 2.9% rate in their non-twin siblings[4]. Further evidence for genetic susceptibility has come from adoption and half-sibling studies[5-7]. Both studies illustrate the importance of genes and were unable to implicate the shared familial micro-environment for the familial aggregation of MS.

Many genome-wide scans for linkage in MS sibling pair families have been undertaken in an effort to identify susceptibility genes[8]. Studies have confirmed the known association with the HLA Class II alleles[9] but evidence for linkage to 6p21 was surprisingly difficult to demonstrate and required many families[10,11]. Non-MHC linkages have been reported, most notably 6q27, 17q24 and 19q13[12-15], but have generally not been replicated in independent samples. Instead of sibling pair families, other research groups have focused on inbred families[16] or large kindreds[17,18] with modest results observed for 9q21, 12p12 and 19q13. However, the power to detect linkage was relatively low as families consisted of 5 to 8 individuals with MS. This is not unexpected as the presence of many affected individuals in a single family is exceptionally rare. For example, in a screen of nearly 30,000 MS patients from across Canada, only 1 family had 11 cases of MS, 1 family had 9 cases, 1 family had 8 cases

and 3 families had 7 family members with MS (Ebers and Sadovnick-unpublished observations).

We have previously presented a family with 14 and possibly 15 affected individuals with MS in 4 generations[19]. Segregation of MS is consistent with an autosomal dominant trait with reduced penetrance. The penetrance is consistent between generations II and III (0.28 and 0.32 respectively) and here we present the results of a genome scan and parametric linkage analysis.

Methods:

Ascertainment:

The family was ascertained in early 1991 and has been evaluated at regular intervals over the last 14 years. Since the time of our initial monograph of the clinical characteristics of this family[19], an additional family member in the fourth generation has become affected with definite MS (Figure 1). The total number of individuals with MS is now 15 and possibly 16 if we include the great-grandfather.

DNA collection:

Samples were collected from eighty-two individuals. The founders (I-1 and I-2) were deceased at the time of sample collection and genotypes were inferred from the genotypes of eleven of their thirteen children. This was also the case for II-11; his 11 children and spouse were used to infer haplotypes. All other affected individuals in Figure 1 were available for DNA collection with the exception of individual IV-7 who was ascertained *after* the genotyping stage of this project during routine biannual follow-up. DNA extraction was by standard protocols[11].

[insert figure 1 here]

***Genotyping*:**

A total of 681 microsatellite markers were genotyped for the genomic screen. The screen was performed in two parts; the first was carried out at the Wellcome Trust Centre (WTC) and the second by the Marshfield Genotyping Service. Two hundred and eighty-three microsatellites from the LMS2 mapping panel (ABI PRISM™ Set Version 2) were genotyped at the WTC. PCR conditions for microsatellite genotyping were as follows: final volume of 10 μl with 50ng of genomic DNA, 10mM Tris-HCL, pH8.3, 50mM KCL, 1.0-3.0 mM $MgCl_2$, 0.60 μmol unlabelled primer, 200μM of each dNTP and 0.25U of Taq DNA polymerase. The markers were genotyped fluorescently and primers were end-labelled with FAM, NED and HEX. Cycle conditions were 94°C for 5 min., 30 cycles of 94 °C for 1 min., 50-62 °C for 1 min., followed by an elongation step for 5 min. at 72° C. Fluorescent products were separated on ABI 373XL (Perkin-Elmer Applied Biosystems). The data from the automated fluorescent platforms was scored using the Genescan 3.1 and the Genotyper software 2.5 (Applied Biosystems). The other genotypes were generated by the Marshfield NHLBI Mammalian Genotyping service (http://research.marshfieldclinic.org/genetics/) with 398 microsatellite markers. CEPH controls were used for consistency of allele calling. Details of this service are given in Weber and Broman[20].

The genotyping of the HLA-DRB1 locus was performed using an allele-specific amplification methodology[21]. A PCR-restriction enzyme digest assay was used to genotype 14 polymorphisms of the TCR β variable region[22].

Markers:

The average distance between the 681 markers was 5.3cM with a range of 0-16.89cM. A total of 603 inter-marker distances were less than 10cM (89%). The distances between the markers were taken from the Marshfield Genotyping Centre "build your own map" function at http://research.marshfieldclinic.org/genetics/ .

Statistical Analysis:

The combination of both genotyping stages required that the map and genotype files be prepared and managed with the program *ShaZam* written by Dr. MZ Cader (Wellcome Trust Centre for Human Genetics-unpublished). The genotype files were checked for inconsistencies using the Pedcheck program[23]. Two-point linkage analysis was performed using the LINKAGE package of statistical programs[24]. Files for multipoint analysis were prepared with MEGA2[25] and parametric linkage and haplotype analysis was performed with SIMWALK 2.0[26].

Linkage was assessed under two models. The first model for analysis was a "*rare dominant disease allele*" model. Parameters were designated under the assumption that the distribution of MS in MS1 is due to a rare mutation present within this single family. The disease allele frequency was set at 0.001 and the phenocopy rate also at 0.001 approximating the population prevalence of MS in Canada[1]. The penetrance was 0.50 for females and 0.20 for males.

The parameters for the second model were for a "*common dominant disease allele"* that included a disease allele frequency of 15%. The model was also "penetrance-free" with the penetrance was set at 0.01 and the phenocopy rate was set at 0.001. Multipoint exclusion mapping was performed for both models with a LOD score of -1 as the cut-off for exclusion[27].

Results

The MHC

The known HLA susceptibility allele is HLA-*HLA-DRB1*15* and this allele is present in 11 of 14 (78.6%) available affected family members. In each instance where at least one parent was positive for *HLA-DRB1*15*, one or more *HLA-DRB1*15* alleles were transmitted to the affected offspring (11/11). A transmission disequilibrium test (TDT) from heterozygous parents was significant for *HLA-DRB1*15* (10 transmissions to 1 non transmission; χ^2=7.36; p=0.0054).

The *un*affected siblings were also assessed for parental transmission of *HLA-DRB1*15*. The 49 unaffected offspring of *HLA-DRB1*15* heterozygous parents received the *HLA-DRB1*15* allele 23/61 (38%) times and did not receive *HLA-DRB1*15* 38/61 (62%) times from their parents; χ^2=3.68, p=0.07. The frequency of HLA-DRB1*15 was 38% (24/65) and was significantly different than the frequency of 78% (11/14)in genotyped cases; χ^2=7.99, p=0.004.

*"Married-in" versus "familial" HLA-DRB1*15*

To further investigate the evidence for linkage in the presence of a strong association, the origin of the *HLA-DRB1*15* allele in the 11 *HLA-DRB1*15*-bearing offspring was assessed in MS1. In four offspring *HLA-DRB1*15* was received from the "familial" line (II-11, -17, III-15, -52) and in 5 other cases *HLA-DRB1*15* was received from the "married-in" spouse (III-24, -28, IV-2,-5,-11). "Familial" is defined as any allele inherited from the founders; "married-in" is defined as those alleles inherited from non-founders marrying into the family. The 2 remaining *HLA-DRB1*15*-bearing offspring (III-38, -39) were both homozygous and received *HLA-DRB1*15* from the "familial" line and the "married-in" line. The "familial" to "married-in" ratio is 6 to

7. Thus the autosomal dominant pattern of inheritance for MS present in this family cannot be attributed to the HLA *HLA-DRB1*15* allele.

Genome-wide linkage analysis- Rare model:

The two-point results of a genome-wide scan analyzed under a "*rare dominant disease allele*" model are presented in Table 1. Eleven markers met the minimum criterion for reporting with $Z_{max} \geq 0.83$. This cut-off level corresponds to an approximate point-wise significance level of 0.05. The highest score observed was for marker D7S559 of 7q36 (Z_{max}=1.34). These results are consistent with chance expectations.

Table 1. Two point linkage results (rare model*)

Cytogenetic location	Marker	Zmax	Recombination fraction (θ)
1p21	D1S206	0.83	0.20
7p21	D7S2200	0.99	0.10
7q36	D7S559	1.34	0.20
9q21	D9S1120	0.84	0.20
9q22	D9S287	0.97	0.20
12p12	D12S373	0.87	0.20
14q11	D14S261	1.07	0.05
17q21	D17S1299	0.89	0.20
17q23	D17S1290	1.02	0.20
19p13	D19S216	1.10	0.01
19p13	D19S884	1.09	0.20

*- For the rare model the disease allele frequency was set at 0.001, phenocopy rate was 0.001 and the penetrance was 0.50 for females and 0.20 for males.

In a multipoint linkage analysis under the *rare* model there was only 1 region with a LOD score over 0.83. The Z_{max}=1.30 at D7S559. This was the most distal marker on chromosome 7q. In total, 99.6% of the genome could be excluded at a LOD<-1 and 96% at LOD <-2 with this *rare* disease model.

Genome-wide linkage analysis- Common model:

There were no two-point LOD scores over 3.3 under a *common* disease allele model. There were also no LOD scores over our arbitrary cut-off $Z_{max}\geq$0.83. The highest score was at 1p21.1 (LOD=0.70; θ=0) for marker GATA133A08. In total there were 12 markers with LOD>0.50 at 1p21, 10q26, 12q24, 19q13, 2q14, 3q26, 5p13, 7p21, 7p15 sand 7q32. The highest two-point score at HLA DRB1 was Z_{max}=0.07 (θ=0.30).

A multipoint analysis of the genotype data was also performed. There were 4 regions that met our minimum criterion with LOD scores over 0.83 (Table 3). The greatest evidence for sharing was seen at 15q14 for marker GATA50C03 (Table 3) - an increase from the Z_{max}=0.07 observed in the two-point analysis. The multipoint Z_{max} at *HLA-DRB1* was -0.366.

Table 3. Multipoint linkage results of MS1 (Common model)

Marker(s)	Cytogenetic location	Multipoint LOD score
D3S2427	3q26	0.89
D4S2431	4q34	0.91
D9S1817	9p13	0.91
GATA50C03	15q14	0.92

Chromosome 7q35-36

In the *rare* model tested the region 7q35-36 was highlighted as another potential susceptibility region in the genome-wide multipoint and two-point analyses. As this region contains the TCR β locus, 14 RFLP's from the TCR β variable region were genotyped within this family and tested for linkage and for transmission distortion. Under the *rare* disease allele model the Z_{max}=0.123 for BV3S1 and when only those MS patients who are *HLA-DRB1*15* positive were counted as affected the Z_{max}=0.410. This stratification based upon DRB1 status was based upon previous findings that TCR β is associated in the presence of *HLA-DRB1*15*[28]. A transmission disequilibrium test was performed and no alleles were observed to be preferentially transmitted to affected offspring. The haplotype BV2S1*1-BV25S1*1-BV26S1*1 showed a trend of association in a Canadian sample of *HLA-DRB1*15* positive sibling

pairs[29]. This haplotype was not present in any of the affected individuals within this family.

Discussion

Extended multiplex families showing an autosomal dominant mode of inheritance have been instrumental in the mapping of disease genes for neurological conditions such as Parkinson's and Alzheimer's disease[30,31]. For these disorders, the majority of cases in the population are generally sporadic and inheritance is seemingly complex. However, identified within these disease populations are loaded families with Mendelian patterns of disease transmission. It is with these families that disease genes have been identified and the insight gained into disease pathogenesis has been invaluable.

The MS family presented here is unique with respect to the number of cases and to our knowledge a family with as many affected individuals has not been reported in the MS literature or that of other autoimmune disorders. The family has been followed for many years and the clinical workup has been exhaustive[19]. The broad clinical spectrum with respect to clinical course and age of onset as well as the HLA association and reduced penetrance supports that MS in this family is the same as that seen in the general MS population.

A key premise to this study is that the observed MS rate is above what we would expect by chance. By following this family for over a decade we are able to address this issue. At the time of initial ascertainment there were 9 cases of MS. Given an annual population incidence of MS of 6 per 100/000 we would expect to see 0.30 individuals to become affected with MS over 14 years. The observation of 6 new cases in this cohort of 354 individuals is a 20-fold increase over what is expected in

the general population and argues in favour of a strong familial component to MS within this single family.

Not only is the prevalence greater than what we would expect, but the mode of inheritance seems to follow an autosomal dominant pattern with reduced penetrance. MS is present in all 4 generations and the penetrance (~30%) is consistent among the respective generations[19]. Despite this apparent Mendelian pattern of inheritance, no significant finding (LOD>3.3) in our genome-wide scan for linkage was observed. The family does have enough power to detect a linked locus[19] and the number of markers and cM coverage used was extensive. This result is therefore puzzling and warrants speculation as to the reason(s) for such negative findings.

Firstly, we may have misspecified the parameters for the linkage analysis. The dominant mode of inheritance appears correct but other variables such as phenocopy rate, locus heterogeneity and common alleles acting as modifiers of risk may all act to confound the analysis and decrease the overall power to identify a linked locus. Alternatively, MS could also be more oligogenic within this single pedigree than is readily apparent, perhaps similar to what is hypothesised in the general MS population. The association with *HLA-DRB1*15* is in keeping with this oligogenic model. However, as for other MS associations, the TCR β haplotype BV25S1*1-BV26S1*1-BV2S1*1 was *not* present in the pedigree. Another candidate, the MS-associated retrovirus, is attractive for its oligogenic nature as it is present in multiple copies on many chromosomes throughout the genome[32,33] and, theoretically could mimic the dominant pattern observed in this family. However an oligogenic hypothesis is difficult to reconcile with the consistent penetrance (≈30%) between the generations and would require that the multiple loci have disease alleles with a common population frequency.

Another explanation may involve the transposition of genetic material. Rearrangements have happened multiple times in human history and can be responsible for human diseases such as familial hypocholesterolemia and acute myeloid leukaemia[34]. If a transposable element–mediated rearrangement were to have occurred, even one time, in this single pedigree it could well confound any traditional linkage analysis. Granted, such genetic instability would have had to escape negative selection pressures and this does not seem to be the case in the general population[35].

One might speculate that this family is an inevitable chance observation. This is unlikely for several reasons. We have screened 28,000 MS cases across Canada as part of the Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS). A striking observation among familial cases was the rapid decrease in number of affected relatives per family. While there were many 2 and 3 affected case families (10%) identified, the number of 4 and 5 member cases was 0.2%, moreover there were only 5 families (out of 28,000) with 7, 8 and 9 members affected in the Canadian population. The observation of a single family with 16 affected is an outlier not following a chance/random distribution. Similarly, consistent penetrance through 4 generations and in the last, developing prospectively over a decade make these most unlikely to be related to chance.

Lastly, it appears that *HLA-DRB1* is the principal genetic locus responsible for MS. While the linkage analysis provided a LOD = 0.07 for *HLA-DRB1*, other data highlights the importance of this region. For example, when *HLA-DRB1*15* was carried by either parent, a DRB1*15 allele was transmitted to the affected offspring 100% of the time (11/11), and only 38% of the time to unaffected siblings (p=0.07). This seems to contrast with other MS families where the rate of under-transmission to

unaffected siblings is not statistically different from 50%[11,36]. There was a significant difference between the frequency of DRB1*15 in the cases (79%) as compared to their unaffected siblings (38%) (p=0.004). Taken together, these findings support the notion that *HLA-DRB1* has a relatively stronger effect within this familial context both in susceptibility and protection. Of the few genotyped MS patients that did not bear *HLA-DRB1*15*, two of the three did bear *HLA-DRB1*04*, a susceptibility allele identified in non-Northern European ethnic groups[37].

We cannot rule out the possibility of an environmental cofactor in this family. Intriguingly, this family of dairy farmers has had no added vitamin D to their intake of locally produced milk over the time spanned by this pedigree.

In conclusion, this single family demonstrates clearly the difficulty of the genetic dissection of complex traits. Common alleles, locus heterogeneity, phenocopies and sample size are but a few factors that can confound genetic analysis in an apparently autosomal dominant family and the absence of any significant linkage prompts reconsideration of environmental cofactors. The study highlights the complexity of MS genetics within a single family seemingly segregating as an autosomal dominant trait.

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