Human Molecular Genetics, 1997, Vol. 6, No. 10 Review 1693–1698

Genetics of multiple sclerosis

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Received July 16, 1997

Multiple Sclerosis (MS) is a common chronic central nervous system disease in young adults. Relative familial risk appears to be determined largely by genes while population risk is strongly influenced by environmental factors. This is supported by genetic epidemiological studies which also suggest an oligogenic inheritance of susceptibility. The HLA DRB1*1501, DQA1*0102, DQB1 0602 haplotype is associated with the disease but HLA contributes only modestly to overall susceptibility. The results of three genomic searches are concordant with the genetic epidemiology and imply a number of genes with interacting effects will be found. Importantly, no single region has been identified with a major influence on familial risk.

INTRODUCTION

Multiple sclerosis (MS) is an excellent paradigm for the investigation of the general category of disorders we now call complex traits. The phenotype is highly variable with symptoms including gait disorder, visual loss, spasticity and fatigue (1). In Canada, the prevalence of MS is ~1/1000 or 0.1% among caucasians of central and northern European origin (2,3). There is also a well recognized preponderance of females which approaches a female to male ratio of 2:1 (4). The average age for MS onset is between 28–30 with females having their first symptom a year or two earlier than males (5).

Although it is very clear now that both genes and the environment must be operative, it has not always been so. Early investigators recognized both an uneven geographical distribution of the disorder in northern European-derived populations and an increased prevalence of the disease as one moves away from the equator (6–9). This served as a pheromone for the epidemiologists who dominated thinking about MS pathogenesis for decades, and led to intense focus on identifying those environmental factors responsible for the observed North/South disease gradients and the reported ‘MS epidemics’ (10–12). The last few decades have seen, in turn, the emphasis on possible analogies between MS and the viral diseases of polio, subacute sclerosing panencephalopathy, progressive multifocal leukoencephalopathy and prion disease. These have yielded somewhat, but by no means wholly, to a spontaneous organ-specific autoimmune disease paradigm best exemplified by spontaneous murine autoimmune disorders of the mouse, such as the non-obese diabetic (NOD) mouse (13,14). There is, however, no convincing spontaneous animal model for MS.

Strong evidence for environmental factors notwithstanding, the shift away from the notion that MS is an acquired infectious disease has been towards the concept that disease susceptibility is determined by genes with as yet unknown interactions among them and with unidentified, but highly important non-heritable factors. In support of this hypothesis is much data from genetic epidemiological research including the observation of resistant ethnic groups residing in high risk regions (15–17), the low rate of conjugal MS (18,19) and the family studies outlined below. While a pattern has developed to indicate inheritance of susceptibility will be important and complex, actual molecular genetic data have been slow to emerge.

FAMILY STUDIES

Proof of familial aggregation does not discriminate between genes and environment, yet it is necessary to explore genetic contributions to disease and familial recurrence data is a good point of departure for assessing any genetic hypothesis. Although absolute recurrence risks have been low in MS for even first degree relatives (only 4% for full-sibs), it has taken population-based studies to get consistent results which can be used to logically extend these observations (20–22).

The empiric risk of having an affected relative with MS approaches 15–20% in regions of the world with high prevalence rates (20). Table 1 lists the recurrence risks for monozygotic (MZ) and dizygotic (DZ) twins, full-sibs and half-sibs, as well as for cousins derived from population-based data (21,23,24). The table demonstrates that a steep drop in risk is observed between monozygotic twins (31%) and first degree relatives (3–5%). The decreases in risk between first degree and second degree relatives, and between second and third degree relatives are less but the data are not immune to ascertainment bias. Taken in sum, the rapid decrease in risk supports the idea of oligo/polygenic inheritance with epistatic interactions among susceptibility loci. We have attempted

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to fit the known $\lambda_{relative}$ values (recurrence risk of relative/population prevalence) to a variety of genetic models and have found that although the observed recurrence risks can be exactly reproduced by several plausible genetic models (Risch, Sadovnick and Ebers, unpublished observations), there is no way, as yet, to discriminate among them. The process is not unlike selecting a number and asking how many ways can one reach this number using a combination of addition, multiplication, subtraction and division.

Table 1. Degree of relatedness and familial risk

Relation to proband	%Sharing	Recurrence risk (%)	λ Value	Reference
Monozygotic twins	100	30.8	308	(23)
Dizygotic twins	50	4.7	47	(23)
Full-siblings	50	3.46	34.6	(24)
Half-siblings	25	1.47	14.7	(24)
Cousins	12.5	0.88	5.9	(21)

TWIN, ADOPTEE, AND HALF-SIB STUDIES

The classical method of distinguishing between genes and environment is the study of twins and several excellent twin studies have been carried out in multiple sclerosis. The similarity of these population-based studies is reassuring in that in all instances the monozygotic twin rate (~30%) exceeds that for dizygotic twins by a factor approaching 10-fold (23,25–29), [one recent volunteer-based study gave a result nearer to 2-fold (30)]. The consensus difference between MZ and DZ twin concordance rates establish that genes are involved in the process of susceptibility.

It has been argued that high concordance in identical twins is, at least in part, accounted for by shared environment. Accordingly, genetic epidemiological efforts have attempted to resolve this and other issues by the study of adoptees and half-siblings. The strategy for adoptee studies is straightforward and in multiple sclerosis the results have been unambiguous (31). Table 2 documents the rate of MS in 1201 *non-biological* first degree relatives of MS patients adopted prior to the age of one and ascertained by population-based screening of more than 16 000 MS patients. Using age-adjusted risks, some 25 affected individuals are expected in a population of biological relatives of this size. In the adoptive sample, only a single individual was affected with MS providing a prevalence rate in this population (constituted rather like the general population with respect to age) of 1 in 1201, remarkably similar to the prevalence rate in the general population from which these individuals were drawn. It was not possible to carry out unbiased ascertainment of the rate of MS in the biological relatives of adoptee probands, but an observed rate similar to that for biological relatives from intact nuclear families independently supports the conclusion from these studies that familial aggregation is very largely or entirely determined by genes (31).

These data are strongly supported by the results of studies from half-siblings (24). Half-sibs are an underutilised and powerful resource for assessing the relative importance of genes and environment in human disease. In addition, they have a particular role in addressing epigenetic phenomena which include vertical transmission of infection, *in utero* effects, breast feeding, genomic imprinting, not to mention mitochondrial inheritance and a host of other differential effects between maternal and paternal nature/nurture. In principle, the studies are straightforward and feasible, given the high frequency in modern populations of marital discord and second and even third families. The estimated maternal and paternal half-sib risks for MS (1.4 and 1.2% respectively, n.s.) indicate the lack of any substantial maternal effect operating in MS pathogenesis. The non-significant difference between the risk to half-sibs who lived with the proband (1.2%) and the risk to half-sibs who never lived with the proband (1.5%) again supports the hypothesis that the familial aggregation of MS is primarily determined by genes and not by a shared familial environment. Nevertheless, it must be emphasized that the *population risk* must be strongly influenced by the environment since populations with similar gene pools can have several-fold differences in risk associated with difference in latitude, cf. Queensland versus Tasmania (32,33). In this respect, the environmental variable appears to be influencing the population as a whole. This variable could be a factor such as climate, diet or some indirect association or consequence of an ubiquitous effect of this nature.

CANDIDATE GENES

The association between MS and HLA polymorphisms is the only consistently replicated molecular genetic result in MS research. Surprisingly, since the time of the first reported association 25 years ago, there has been relatively little refinement in identifying a causative allele. The first studies demonstrated an association with the HLA Class I antigens A3 and B7 (34–36). Following these initial reports, HLA was shown to be associated with the Class II polymorphisms Dw2 and DR2 (37,38). This has been sub-typed into a strong and consistent association with the HLA DRB1*1501, DQA1*0102 and DQB1*0602 haplotype (39–41). The strong linkage disequilibrium present within this region makes elucidation of a specific susceptibility allele difficult (42). Additionally, the association with the DR/DQ haplotype does not exclude the possibility that other genes in the region are also playing a role in MS pathogenesis. There have been observations suggesting the presence of HLA loci operating in *cis* and/or *trans* with DRB1*1501, DQA1*0102, DQB1*0602 to increase relative risk (43–45), the possibility of genetic heterogeneity (46) and even the presence of resistance alleles (47,48). However, these data are short of being definitive. Taken together, HLA is most certainly involved in the process of susceptibility, yet given the relatively weak results of linkage analysis (49–51), it can account for but a small portion of overall susceptibility. (The excess of haplotype sharing in this region is only slightly in excess of that expected by chance.)

Table 2. Expected versus observed number of non-biological relatives with MS

Non-biological relatives	Age-adjusted		Poisson probability
	Expected	Observed	
Parents (n = 470)	9.2	1	0.0010
Siblings (n = 345)	10.7	0	2.3×10^{-5}
Children (n = 386)	5.5	0	0.0041
Total (n = 1201)	25.4	1	2.5×10^{-10}

Taken from (31)

Table 3. Selected findings using the candidate gene approach

Negative results	Further investigation	Positive results
TCR alpha (60–62)	Immunoglobulin variable region (78)	DRB1*1501-DQA1*0102-DQB1*0602 (82,83,39,40)
Interleukin-1 receptor agonist, Interleukins 1, 2, and Interleukin 2, 5 receptors (63,64)	MBP (76,77)	
INF α, β, γ (64)	TCR beta (79–81)	
Immunoglobulin constant region (65–67)		
Alpha-1 anti-trypsin (68)		
C3, C4, BF, C2 (69,70)		
TNF (54–56)		
TAP, LMP (71,72)		
HLA-DP region polymorphisms (73,74)		
Mitochondrial genes (75)		

Tumour necrosis factor (TNF) polymorphisms (tightly linked to the DR/DQ loci) may also be potential candidates for MS susceptibility alleles. The evidence has come from research investigating the role of TNF in the CNS lesions of MS patients and the increased levels of TNF observed in cerebrospinal fluid and sera (52,53). A recent clinical trial in MS employing soluble TNF receptor had a striking effect on disease activity (in preparation), supporting the hypothesis that TNF is an important player in the pathogenesis of an MS lesion. Nevertheless, molecular studies have so far failed to demonstrate a significant association with the TNF locus that is independent of the known HLA Class II associations (54–56).

Because of the strong circumstantial evidence that MS is an autoimmune disorder, the evidence that T cells are activated, that immunoglobulins are produced at the site of the lesions and that a variety of cytokines are released in lesions, other candidate genes involved in these functions have been assessed in individual MS patients compared with controls and in families. Many of the early study populations were not carefully selected and have been criticized on methodological grounds (57). More recently, the use of the transmission disequilibrium test (TDT) and affected family-based controls (AFBAC) has made it easier to discard potential candidates (58,59). Convincing negative results have been obtained for T cell receptor α, interleukin-1 receptor agonist, interferon α, β and γ and a variety of complement and cytokine and enzyme loci (Table 3).

The results are less clear-cut for three reasonable candidates including the myelin basic protein gene (coding for the major myelin protein with which an animal model of MS can be induced), the T cell receptor β locus and immunoglobulin variable gene loci. In the Finnish population, there is evidence that myelin basic protein seems to be both associated and linked with multiple sclerosis (76,77). Nevertheless, it would appear that allele frequencies differ even within the genetically restricted Finnish population and again some confirmation of this result would be welcome, as several other investigators have been unable to find either association or linkage for this locus in more mixed populations (83–85). The T cell receptor β locus is similar in that many studies have found no evidence for association or linkage (87–89); however, positive results have been reported by more than one group. In one case, linkage was reported to the locus directly, and in others evidence for linkage was found when the population was stratified by HLA type, an association being found for those who were DR2 positive but not for those who were DR2 negative (79–81). Another potential susceptibility locus may lie within the immunoglobulin variable gene region. A positive association with a VH2-5 allele of the variable gene region was observed (65,78), though an affected sib-pair analysis was negative (78).

Table 4. Genome screens—markers showing positive results (MLS>0.50)

Full genome screens			Syntenic regions to EAE loci (94,95)
American (91)	British (92)	Canadian (90)	Finnish (93)
	D1S199 (1.2)		
D1S236 (1.5)	D1S201 (0.93)		
D2S131 (1.71)		D2S119 (1.24)	
D2S155 (0.69)			
D3S1744 (1.0)		D3S1261 (0.99)	
D3S1309 (1.01)			
D4S1566 (0.81)		D4S431 (0.68)	
D4S402 (0.60)			
D5S815 (1.14)	D5S428 (1.1)	D5S406 (4.24)	D5S416 (3.40)
D6S273 (3.57)			
D6S1693 (0.64)	D6S276 (2.8)		
D6S308 (1.0)	D6S461 (0.65)		
D7S554 (2.86)			
D7S523 (1.11)	D7S516 (1.7)	D7S513 (0.87)	
D7S524 (0.70)			
D9S162 (1.24)			
D9S9566 (1.13)			
D10S464 (1.39)		D10S212 (0.97)	
D11S922 (1.13)		D11S200 (1.38)	
D12S1052 (1.48)			
D13S285 (0.87)			
	D14S292 (1.4)		
D16S748 (1.75)			
	D17S798 (1.6)		
D17S942 (2.7)			
D18S66 (0.93)		D18S59 (0.56)	
APOC2 (1.47)	APOC2 (0.90)	D19S47 (0.73)	
	DXS991 (1.8)	DXS1068 (1.85)	

( ) refers to the maximum lod score attained for the marker.

Table 5. Regions of overlap between full genomic screens

American (91)	British (92)	Canadian (90)
	1p36-p33	1p36-p33
2p23	2p23-p21	2p23-p21
	3p14-p13	3p14-p13
3q22-q24		3q22-q24
4q31-qter	4q31-qter	
5q13-q23	5q12-q13	5q12-q13
6p21	6p21	6p21
6q27	6q22-q27	
7q11-q22		7q21-q22
18p11		18p11
19q13	19q12-q13	19q13

GENOME SCREENS

Given the lack of success of the candidate gene approach in identifying susceptibility genes for multiple sclerosis, a number of groups have turned to full genome searches using widely available and highly informative microsatellite polymorphisms and large numbers of affected sib pairs (90–92). Table 4 gives a chromosome by chromosome accounting of positive results (defined somewhat differently for each study). Because each screen used somewhat overlapping but often different sets of markers, it is not easily possible to produce a composite of the results. However Table 5 gives some idea of where regions of common interest suitable for hypothesis generation and further testing are located. Taken in sum, the screens provide strong evidence of exclusion of a major locus contributing a λ_{sibs} value >3 for 88% of the genome for the Canadian study, 92% of the genome for λ_{sibs} value >5 in the British study. Positive scores were found (although not necessarily for the same marker) in all three studies for chromosomes 2p23, 5q13, 6p21, and 19q13. A fourth group performed a genomic screen of regions syntenic to the susceptibility alleles identified in the EAE mouse model of MS (93). It must be emphasized that the results of the screens are to be interpreted in the context of what we predict will be an erroneous supposition, i.e, that all MS families have their susceptibility determined by the same loci within (and between) populations. If heterogeneity is common in Mendelian disorders, to what extent can we expect complexity/heterogeneity in a complex trait?

There is already mounting evidence for heterogeneity or perhaps better stated in the context of MS, complexity. If indeed DR or DQ alleles singly or in combination code for susceptibility, it must be recalled that many patients fail to bear these alleles, and the relative lack of haplotype sharing suggests that the MHC contributes <10% of the overall genetic susceptibility to this disease (96). It would not be surprising that MS patients will differ for the relative or absolute importance of specific loci in individual or familial susceptibility. Stratification of patient samples by phenotype might increase homogeneity, but enthusiasm for this approach must be tempered by the high intrafamilial variability of clinical course. Also, studies examining HLA polymorphisms and clinical phenotype have provided conflicting results (45,47,97,98).

Complexity is also strongly implied by the observation that the relatives of concordant twins have a higher risk than do the relatives of discordant twins (G.C. Ebers, A.D. Sadovnick, D.A. Dyment, unpublished data), consistent with a threshold/oligogenic model. Similarly, age of onset and recurrence risk in other relatives appear to be independent factors influencing recurrence risk in the first degree relatives of MS probands (99), and additionally, there is an increased risk of MS for the offspring of conjugal pairs (100). Again, these results are consonant with a model in which susceptibility is determined by a number of loci with susceptibility alleles in excess of that which is required or necessary to account for the development of the disease being present in those with early onset or having affected relatives.

THE FUTURE

MS is a compelling problem, often striking in the prime of life when education has ended and both familial and societal responsibilities have been assumed. Scientifically, the evidence that MS is a complex trait is definitive. We are now at a point when several groups have the resources available to identify the chromosomal regions involved in the processes of susceptibility. There are likely to be several regions harbouring susceptibility alleles and the contribution by the HLA locus only accounts for perhaps 10% of familial risk. In addition to the identification of chromosomal regions and specific susceptibility genes, the elucidation of the environmental components to MS pathogenesis will be important. These non-heritable factors remain largely inscrutable, yet should not be ignored. By understanding the genetic–environmental interactions it may be possible to devise new therapies to better treat MS patients and to test preventive strategies aimed at decreasing the occurrence of this common disorder.

ACKNOWLEDGMENTS

We would like to thank and acknowledge the helpful discussion of Holly Armstrong and Neil Risch. The authors would also like to thank Cheryl Johnson, Debra King and Marian Young for their valuable assistance in the preparation of this manuscript.

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