



## **Analysis of defects or variants in individual genes as a possible cause of Multiple Sclerosis**

Draft: October 26, 2006  
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### **Summary**

Genetic variations that lead to disease vary greatly in scale, ranging from single base pair alterations to deletions or duplications of entire chromosomes. The lack of evidence to date linking risk of MS with defects covering large chromosomal segments makes it likely that the genetic underpinnings of MS involve smaller variants that affect individual genes. However, it is not yet known what physical types of genetic variations are involved – such as point mutations, insertions, or deletions – and in what genes they are located. So far the hundreds of studies attempting to pinpoint the specific genes and variants that influence risk of MS have produced mostly inconclusive evidence. Since phenotypic evidence pointing to a particular type of defect has not been documented in MS, determining the physical nature of the variants involved will likely come only upon further success in identifying the MS susceptibility genes themselves.

### **Hypothesis**

Genetic susceptibility to MS is determined by defects or variants affecting individual genes, as opposed to large-scale chromosomal defects.

### **Experimental tests of the hypothesis**

Analyses of high-risk populations and families affected by MS strongly indicate that inherited genetic factors play a role in determining susceptibility to the disease. These factors could be either large-scale in nature, spanning multiple genes, extended chromosomal regions or even entire chromosomes, or relatively small in scale, involving only individual genes. At this time it seems improbable that inherited large-scale chromosomal defects are widely involved in increasing risk to MS (see our document, "Analysis of chromosomal abnormalities as a possible cause of Multiple Sclerosis"). Therefore defects or variants in individual genes most likely constitute the heritable component to MS risk; in addition, somatic changes to individual genes may further influence MS susceptibility.

There are many physical types of variants that affect individual genes, including point mutations or substitutions, insertions, deletions, inversions, and duplications. For the most part, these variant types do not manifest any uniquely identifiable feature. A few defect types do exhibit distinguishing characteristics that might suggest their involvement in a disease (for instance, triplet repeat expansions often produce the phenomenon of

anticipation, in which later generations in affected families experience an earlier age of onset), but studies of MS phenotype and familial patterns have not linked any of these characteristics with MS. (An anticipation effect was recently found in a cohort of Sardinian MS subjects, but this was deemed to be the result of environmental, not genetic, influences<sup>1</sup>.) Therefore, any of the defect types listed above should be considered candidates for increasing the risk of MS.

Identifying the specific mutations or variations involved in MS will first necessitate locating the susceptibility genes or regions themselves, an effort which is still in progress. (See our analyses of alleles on the autosomal chromosomes, X or Y chromosomes, and mitochondrial DNA to learn about the status of these efforts.) Once susceptibility genes have been determined, then scientists can start to distinguish the specific risk-enhancing alleles in those genes.

Identification of susceptibility alleles is currently underway for the genetic region that has demonstrated the most consistent association with MS to date, the HLA (human leukocyte antigen) region on chromosome 6. The HLA region is characterized by extensive polymorphism (in particular, numerous functional substitutions), leading to numerous structural differences in class I and II molecules between individuals. It is conceivable that HLA variants influence risk of MS by shaping an individual's immune response to particular antigens.

Having repeatedly demonstrated a potential relationship between MS and HLA variants, scientists are now striving to determine the specific alleles that influence susceptibility to MS. One complicating factor in identifying the specific variants that increase MS susceptibility is the high degree of linkage disequilibrium in this region which has made it difficult to identify the particular allele or alleles that increase risk of MS. For instance, the DR2 haplotype which has shown strong association with MS includes multiple alleles such as DRB1\*1501 and DQB1\*0602. However, this issue is now being addressed through techniques such as the study of populations with less extensive linkage disequilibrium. For instance, a recent study of the DR2 haplotype in African-Americans was able to detect a primary contribution of DRB1\*15 to MS risk distinct from the DQB1 locus, which was not found to independently influence risk<sup>2</sup>. Other studies are exploring interactions between alleles in the HLA region that may increase or decrease the risk of MS<sup>3</sup>. Another approach that is being taken is to perform functional studies of MS-associated HLA alleles to assess their ability to present particular antigens; these may further help identify the particular genetic sequences and substitutions that appear most important in predisposing to MS<sup>4,5</sup>. For example, one recent study described a T cell clone that can present over 30 different peptides to each of the DR2 DR and DQ molecules, indicating functional redundancy among these molecules and suggesting that the combination of alleles in the DR2 haplotype may be important in MS etiology<sup>6</sup>.

## **Conclusions**

The lack of strong evidence implicating chromosomal aberrations in the etiology of MS makes it likely that the genetic cause(s) of MS are to be found within one or more individual genes as opposed to large blocks of contiguous genes. Indeed, one of the leading hypotheses today regarding the genetic nature of MS is that MS susceptibility is influenced by several genes, and that the specific variations in question may even be common alleles that are nonpathogenic except when combined with certain other genetic or environmental factors.

Genetic variants that affect single genes include point mutations or substitutions (classified as silent, missense, nonsense, or splice site abnormalities), deletions, insertions, inversions, and duplications. A few classes of variants provide phenotypic clues about their physical nature; however, MS does not exhibit any phenotypic characteristics that would indicate the involvement of one type of variant over another. Therefore, identifying the variant(s) involved in MS will likely depend on identifying the susceptibility gene(s). The research conducted to date to locate MS genes has not yet provided a great deal of evidence linking particular polymorphisms to risk of MS. Most of the functionally significant polymorphisms studied have produced either negative, conflicting, or unconfirmed positive results. Those polymorphisms more concretely linked with MS (such as those in the HLA DR2 haplotype) have yet to be completely distinguished from other genetically linked variants as true risk factors, although for certain HLA variants this issue is currently being addressed. Further work is needed to determine the genes involved in MS and understand which specific variants are critical; the results of these efforts will give us deeper insights into the development of MS and lead to other benefits in areas such as therapy development.

## References

- 1 E. Cocco, C. Sardu, M. Lai, G. Spinicci, P. Contu and M. G. Marrosu, *Anticipation of age at onset in multiple sclerosis: a Sardinian cohort study*. *Neurology.*, 2004. **62**(10): p. 1794-8. PubMed ID: 15159480.

Analysis of the age of onset of MS in a Sardinian cohort revealed that onset in younger MS subjects occurred at a significantly earlier age than in older subjects. No evidence was found to suggest that this effect was due to genetic influences; instead, environmental changes are believed to underlie this phenomenon.

- 2 J. R. Oksenberg, L. F. Barcellos, B. A. Cree, S. E. Baranzini, T. L. Bugawan, O. Khan, R. R. Lincoln, A. Swerdlin, E. Mignot, L. Lin, D. Goodin, H. A. Erlich, S. Schmidt, G. Thomson, D. E. Reich, M. A. Pericak-Vance, J. L. Haines and S. L. Hauser, *Mapping Multiple Sclerosis Susceptibility to the HLA-DR Locus in African Americans*. *Am J Hum Genet*, 2003. **74**(p. 1. PubMed ID: 14669136.

Case-control and family-based association studies of African Americans with MS identified a primary role for the HLA-DRB1 locus in increasing susceptibility to MS, and revealed that the DQB1 locus does not independently influence MS risk. The association with DRB1 does not appear to be an artifact of genetic admixture.

- 3 D. A. Dyment, B. M. Herrera, M. Z. Cader, C. J. Willer, M. R. Lincoln, A. D. Sadovnick, N. Risch and G. C. Ebers, *Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance*. *Hum Mol Genet.*, 2005. **14**(14): p. 2019-26. Epub 2005 Jun 1. PubMed ID: 15930013.

A detailed analysis of genotypes in the HLA region was performed in 4,347 individuals from 873 multiplex MS families. In addition to identifying risk-conferring (DR\*15 and DR\*17) and protective (DR\*14) haplotypes, the analysis also revealed interactions between haplotypes that appear to alter the risk of MS.

4 E. Quelvennec, O. Bera, P. Cabre, M. Alizadeh, D. Smadja, F. Jugde, G. Edan and G. Semana, *Genetic and functional studies in multiple sclerosis patients from Martinique attest for a specific and direct role of the HLA-DR locus in the syndrome*. Tissue Antigens, 2003. **61**(2): p. 166-71. PubMed ID: 12694585.  
A study of non-Caucasians (Martinicans) with MS found an association with DRB1\*15 but a neutral role for DQB1\*0602. Structural analysis showed that DRB1\*1501 and \*1503 molecules have similar abilities to present the MBP 85-99 peptide to T cells.

5 H. L. Lang, H. Jacobsen, S. Ikemizu, C. Andersson, K. Harlos, L. Madsen, P. Hjorth, L. Sondergaard, A. Svejgaard, K. Wucherpfennig, D. I. Stuart, J. I. Bell, E. Y. Jones and L. Fugger, *A functional and structural basis for TCR cross-reactivity in multiple sclerosis*. Nat Immunol, 2002. **3**(10): p. 940-3. PubMed ID: 12244309.

Analysis of two HLA DR2 alleles (DRB1\*1501 and DRB5\*0101) revealed that both may influence MS susceptibility. T cell recognition studies and crystal structure analysis demonstrated similarities between a DRB5\*0101-EBV peptide complex and a DRB1\*1501-MBP complex, suggesting molecular mimicry as a cause of MS.

6 M. Sospedra, P. A. Muraro, I. Stefanova, Y. Zhao, K. Chung, Y. Li, M. Giulianotti, R. Simon, R. Mariuzza, C. Pinilla and R. Martin, *Redundancy in antigen-presenting function of the HLA-DR and -DQ molecules in the multiple sclerosis-associated HLA-DR2 haplotype*. J Immunol., 2006. **176**(3): p. 1951-61. PubMed ID: 16424227.

In vivo-expanded CSF-infiltrating T cell clones were shown to use multiple HLA class II molecules as restriction elements. One T cell clone could recognize over 30 peptides presented by each DR and DQ molecule of the DR2 haplotype. Functional redundancy among the different DR2 molecules may facilitate T cell activation and thereby promote the strong T cell response seen in MS.