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# Genetic risk factors in multiple sclerosis and approaches to their identification

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**Development of multiple sclerosis (MS) is believed to involve genetic as well as environmental factors. A complicating aspect to the study of aetiological factors in MS concerns the possible existence of genetically different subtypes of the disease. In addition, a relatively large number of susceptibility genes could be involved. Most likely, the contribution of the single genes to the susceptibility to MS is modest. However, interactions between different genes could result in a dramatic increase in disease susceptibility (synergistic gene effects). In this short review we focus upon genetic heterogeneity and gene interactions in MS. We also outline approaches to the genetic analysis of complex disease traits such as MS.** *Journal of NeuroVirology* (2000) **6,** S23–S27.

**Keywords:** multiple sclerosis; genetic heterogeneity; gene interaction; genetic analyses

## Introduction

Multiple sclerosis (MS) is classified as a complex disease. Development of this group of diseases, most likely, involves interactions between susceptible genotypes and environmental factors. A number of genes have attracted interest as possible susceptibility factors in MS. They can be divided into three main groups: genes affecting immune functions, myelin structural genes and mitochondrial genes. Many genes have been associated with MS but most often the findings have not been confirmed by examinations in other populations. An exception to this are findings that certain haplotypes of HLA-DR and -DQ alleles confer an increased risk of attracting MS, at least in some parts of the world.

## Aetiological and genetic heterogeneity in MS

The varied clinical picture of MS has led to speculations that the disease is not a well-defined aetiological entity but consists of genetically different subtypes in addition to subtypes with no genetic contribution (phenocopies). A complicating aspect is that some genes could be involved in the induction of a disease and others in its progression. Consequently, the genes modifying the severity and course of MS are not necessarily identical with those acting by increasing susceptibility to the disease. There is evidence that MS is associated with different HLA genes in different populations (Table 1). In most European Caucasians and their descendants in North America, Australia and New Zealand, the disease is associated with the serological specificity HLA-DR2 – more precisely with the haplotype DRB1*1501-DQA1*0102-DQB1*0602 (e.g. Allen *et al*, 1994) – but the strength of this association varies between populations. In the Mediterranean region MS has been associated with DR2 as well as DR4 (Saruhan-Direskeneli *et al*, 1997). A similar finding has been done in the Canary Islands (Coraddu *et al*, 1998). Evidence of an association between MS and DR4 has also been found in Arabs (Kurdi *et al*, 1977), whereas the disease appears to be associated with DR2 in the Jewish population of Israel (Kwon *et al*, 1999). In the mainland of Italy, associations of MS with DR2, DR3, DR4 and DR5 have been reported but some of these findings are perhaps influenced by an appreciable degree of ethnical heterogeneity (La Mantia *et al*, 1990 and references therein). A more recent study failed to detect association between MS and DR2 alleles in a population of continental Italians (Ciusani *et al*, 1995). In Sardinia MS has been associated with DR3 and DR4 (Marrosu *et al*, 1997). Two clinically different forms of MS seem to be present in Japan, i.e. a DR2-associated Western type characterised by numerous brain lesions and an optico-spinal or Asian type, which is not associated with any DR type (Kira *et al*, 1996). Finally, in some populations no

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**Table 1** HLA class II markers associated with multiple sclerosis in different parts of the world

| | *Odds ratio (95% CI)*[a] | *Reference* |
|---|---|---|
| *DR2*[c] | | |
| Iceland | 2.40 (1.46–3.93)[b] | Haegert *et al*, 1996 |
| Sweden | 3.48 (2.31–5.25)[b] | Allen *et al*, 1994 |
| Russia | 2.13 (1.40–3.23) | Sudomoina *et al*, 1998 |
| United Kingdom | 4.98 (2.66–9.31) | Kelly *et al*, 1995a |
| Northern Italy | 2.08 (1.09–3.94) | Casetta *et al*, 1991 |
| Northern Spain | 2.78 (1.61–4.81) | Clerici and Fernandez (1992) |
| Canary Islands | 3.86 (1.38–10.76) | Coraddu *et al*, 1998 |
| Turkey | 3.73 (1.59–8.73) | Saruhan-Direskeneli *et al*, 1997 |
| Canada (French Canadians) | 4.49 (2.19–9.20) | Haegert and Francis, 1992 |
| Australia | 6.47 (3.36–12.46) | Stewart *et al*, 1997 |
| Japan (Western-type MS) | 4.24 (1.79–10.07) | Kira *et al*, 1996 |
| India | 2.33 (1.08–5.03) | Kelly *et al*, 1995b |
| Palestine (Arabs) | 5.82 (2.07–16.37) | Al-Din *et al*, 1990 |
| Sicily | 3.39 (1.63–7.05) | Elian *et al*, 1987 |
| *DR3* | | |
| Sardinia[d] | 1.77 (1.28–2.45)[b] | Marrosu *et al*, 1997 |
| Malta | 4.17 (1.16–14.94) | Elian *et al*, 1987 |
| Northern Italy | 2.39 (1.38–4.15) | La Mantia *et al*, 1990 |
| *DR4* | | |
| Canary Islands | 2.97 (1.11–7.95) | Coraddu *et al*, 1998 |
| Sardinia[e] | 1.96 (1.22–3.15)[b] | Marrosu *et al*, 1997 |
| Turkey[e] | 2.91 (1.39–6.09) | Saruhan-Direskeneli *et al*, 1997 |
| *No HLA association* | | |
| Shanghai, China | – | Kelly *et al*, 1995a |
| Hong Kong, China | – | Yu *et al*, 1989 |
| Kuwait | – | Al-Din *et al*, 1990 |
| Japan[f] | – | Kira *et al*, 1996 |

[a]Odds ratios and 95% confidence intervals (95% CI), based upon phenotypes, were calculated using Woolf's method. All calculations were done with the Instat® program (GraphPad Software Inc, San Diego, CA, USA). [b]In a few cases calculations of odds ratios were based upon alleles or haplotypes; [c]The DR2 haplotype associated with MS is DRB1*1501-DQA1*0102-DQB1*0602; [d]The DR3 haplotype associated with MS in Sardinia is DRB1*0301-DQA1*0501-DQB1*0201; [e]The DR4 haplotypes associated with MS in Sardinia and Turkey are, DRB1*0405-DQA1*0501-DQB1*0301 and DRB1*04-DQA1*0301/2-DQB1*0302, respectively; [f]Asian-type MS has recently been associated with DPA1*0202 and DPB1*0501 alleles (Ito *et al*, 1998).

associations or weak associations between MS and HLA genes have been found (Kelly *et al*, 1995a,b). Collectively, these findings suggest that different predisposing genes and perhaps environmental factors as well are responsible for the development of MS in different populations.

Also observations of mitochondrial DNA (mtDNA) suggest presence of genetic heterogeneity in MS. Apparently, an mtDNA haplogroup acts as a risk factor of subsets of MS characterised by visual impairment and optic neuritis although this haplogroup does not appear to contribute to MS susceptibility as such (Reynier *et al*, 1999). Also previous findings have suggested existence of an association between mtDNA and a subgroup of MS patients with visual impairment (Kellar-Wood *et al*, 1994).

Assuming that the genetic background of MS differs between populations, the question arises whether there is also intra-population heterogeneity with respect to susceptibility genes of this disease. The observations from Japan suggesting two clinically distinct MS subtypes seem to support this with DR2 being a marker of one of these subtypes. Our hypothesis is that the genetic susceptibility factors of MS varies between different populations and also within the same population, forming a continuum of diseases with shared and nonshared genetic risk factors. If so, this would obviously complicate a classification of the disease into well-defined genetic subtypes. Another aspect concerns the possible occurrence of phenocopies.

## Gene interactions

Susceptibility to MS is believed to depend upon a number of genes each with moderate effects that perhaps are enhanced through interactive effects. Previous observations of gene interactions in the development of MS have suggested a moderate effect of a T cell receptor (TCR) haplotype in DR2-positive individuals, perhaps representing an additive effect (Beall *et al*, 1989). However, more recent observations did not find evidence in support of interactive effects between DR2 and TCR alleles on susceptibility to MS (Vandevyver *et al*, 1994).

A recent study examined genes regulating apoptosis in patients with systemic lupus erythematosus (SLE), assuming presence of a defect in the apoptotic elimination of autoreactive lymphocytes in this disease (Mehrian *et al*, 1998). Evidence was provided that a *bcl-2* and an *IL-10* allele each increased susceptibility to this disease moderately, whereas the combined presence of both these two alleles resulted in a dramatically increased SLE susceptibility suggesting a synergistic effect (Table 2). Similar strong synergistic gene effects are likely to be involved in the development of MS but have not yet been discovered.

## Approaches to the detection of susceptibility genes

There are several approaches to identify disease genes. One of these approaches is based upon linkage. This term refers to the tendency of two loci

**Table 2** Synergistic effect of *IL-10* and *bcl-2* on systemic lupus erythematosus

| IL-10[a] | *bcl-2*[b] | *Number of individuals* Controls | Cases | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| x/x | y/y | 161 | 87 | 1.00 | – |
| 127/x, 127/127 | y/y | 31 | 27 | 1.61 | 0.90–2.87 |
| x/x | 193/y | 18 | 20 | 2.06 | 1.03–4.09 |
| 127/x, 127/127 | 193/y | 1 | 22 | 40.71 | 5.30–307.2 |

[a]x denotes any other *IL-10* allele than 127; [b]y denotes any other *bcl-2* allele than 193. Modified from Mehrian *et al*, 1998.

on the same chromosome to be inherited together. Linkage analysis is an important tool for mapping of disease genes in families with one or more diseased members. With this type of genetic analysis, chromosomal regions harbouring disease genes can be identified using a panel of microsatellite markers in a genome-wide screen (e.g. Chataway *et al*, 1998). Linkage analysis permits isolation of hitherto unknown genes without requiring knowledge of the pathophysiological mechanisms of the disease under examination. A draw-back of linkage analyses is that they are not reliable for detection of genes with moderate or minor effects. Moreover, they are susceptible to aetiological heterogeneity, i.e. a problem of relevance to the study of genetic risk factors in MS. Finally, a model has to be specified, i.e. assumptions regarding the mode of inheritance and penetrance of the disease as well as other parameters have to be made. Misspecification of the model may have detrimental effects such as reduction in the power to detect linkage. A model-free approach for analysis of linkage is based upon affected sib-pairs, but since this type of analysis lacks statistical power, a large number of sib pairs is necessary.

Linkage analysis has been successfully applied for mapping of disease genes in Mendelian disorders. However, application of this type of analysis for genetic dissection of complex diseases such as MS is a challenge. Several genome-wide linkage studies have been conducted in the search of MS susceptibility genes. They have identified candidate regions possibly harbouring MS susceptibility genes but the disease genes residing in these regions remain to be isolated and characterized (e.g. Chataway *et al*, 1998).

Association studies compare allele frequencies of a specific gene (a candidate gene) in cases and control subjects. Population-based association studies of unrelated patients and control subjects, are usually uncomplicated to conduct but the results achieved by this type of study have to be interpreted with caution due to a risk of false positive associations. A common cause of such spurious associations is population stratification. In order to avoid this, factors such as ethnic and geographical origin of cases and controls are important to consider. Even with a careful matching of cases and control subjects, spurious disease-allele associations occasionally emerge (hidden stratification). Fortunately, this problem can be avoided by the use of intra-familiar association studies with relatives as control subjects. Different types of relatives can be used, including unaffected sibs. Another approach is to pool the untransmitted alleles of the parents of patients and use this pool as the control sample (Terwilliger and Ott, 1992).

A founder population is a type of population derived from a small number of individuals (founders). In such a type of population, individuals with a genetic disease are assumed to have inherited their disease genes from a common ancestor. Founder populations are very suitable for mapping of disease genes. For example, the repertoire of loci involved in a polygenic disease is smaller (less heterogeneity) than in general and mixed populations due to genetic drift. This facilitates their detection but also implies that different susceptibility genes may be identified in different founder populations. Recently, linkage disequilibrium (LD) mapping, also known as identity by descent mapping, was introduced as a tool of mapping disease genes in founder populations (Houwen *et al*, 1994). Using microsatellite markers in a genome-wide search, regions harbouring predisposing genes can be identified in patients by sharing of marker alleles. This differs from the conventional population-based association studies in that control subjects are not included. Of importance aetiological heterogeneity is not detrimental to LD mapping (*cf*. linkage mapping) making this method attractive for genetic dissection of complex diseases such as MS.

Since genetic material is shuffled between homologous chromosomes during meiosis, the age of the population in question (i.e. the number of meioses since its founding) is an important parameter to consider in the design of mapping studies based upon LD. In populations of relatively recent ancestry, the shared chromosomal segments are large as opposed to older populations where the extent of LD is limited. Consequently, marker spacing depends upon the age of the population under examination.

Another method of population-based association/LD mapping compares microsatellite allele frequencies between cases and controls on a genome-wide basis (Barcellos *et al*, 1997). For this purpose a founder population, would be ideal. However, this type of study can also be conducted with cases and controls from general and mixed populations. In the latter case, LD usually does not extend beyond 0.5 – 1.0 cM, implying that marker spacing has to be very dense, e.g. at least 3000 markers corresponding to an average marker interval of 1.0 cM or less. Analysis of this high number of

markers, necessitates pooling of DNAs from patients and control subjects, respectively. For example pool sizes of 100 are feasible (Barcellos *et al*, 1997). Subdivision of patients by relevant criteria prior to pooling such as age of onset, permits a suspicion of aetiological and genetic heterogeneity to be examined. Using fluorescence-based automated detection devices, peak heights can be translated into allele frequencies. However, amplification artifacts (stutter bands), which is a problem adherent to dinucleotide microsatellites, may interfere with the calculation of allele frequencies and perhaps necessitates mathematical correction of the values. An alternative is to use tri- or tetranucleotide microsatellites when possible as they are generally easier to score.

## Conclusion

Research into the aetiology of MS is associated with a number of difficulties, including possible genetic and aetiologic heterogeneity. With the advent of new genetic approaches we will hopefully gain new insights into the aetiology of the disease.

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