## ARTICLE

# Linkage analysis in multiple sclerosis of chromosomal regions syntenic to experimental autoimmune disease loci

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**Multiple sclerosis is a demyelinating disorder of the central nervous system with a putative autoimmune aetiology in which several genes are thought to be involved. Four published genomic screens have confirmed that a gene influencing MS resides within or close to the HLA class II region in 6p21. Still, this locus is likely to confer only a part of the genetic susceptibility in MS. Further, all four studies identified a number of other regions with possible linkage. We have investigated eight chromosomal intervals syntenic to loci of importance for experimental autoimmune model diseases in the rat in 74 Swedish MS families. Possible linkage (a non-parametric linkage NPL score of 1.16 by GENEHUNTER computer package) was observed with markers in 12p13.3, a region syntenic to the rat Oia2 locus which is importance for oil induced arthritis (OIA). Four markers in the T cell receptor $\beta$ chain gene region in 7q35 showed possible linkage (highest NPL score of 1.16). This locus is syntenic to the rat Cia3 locus (collagen induced arthritis). These two loci at least partially overlap with chromosomal regions showing indicative evidence for linkage in the previous MS genomic screens. Indeed, both Oia2 and Cia3 were recently found to be linked also with experimental autoimmune encephalomyelitis, a commonly used model for MS. Markers in 2p12, 3p25, 10q11.23, 17q21-25, 19q13.1, and 22q12-13 failed to provide evidence for linkage. We conclude that evidence is amounting that 12p13-12 and 7q34-36 may harbour genes with an importance for MS. The synteny with experimental loci may eventually facilitate their identification.** *European Journal of Human Genetics* (2001) **9**, 458 – 463.

## Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterised by damage to axonal myelin sheaths and axons, commonly resulting in progressive neurological disability beginning in early adulthood. MS is most prevalent in populations of northern European descent.[1] Twin and adoption studies provide strong evidence for a genetic contribution to the susceptibility of MS.[2]

It is well established that one gene influencing MS is located within or close to the HLA class II gene region.[3] This is consistent with the notion that MS is a T cell-mediated autoimmune disease, and, therefore, other genes controlling the immune response have been studied as potential susceptibility factors in MS. However, results have been inconsistent (for a review, see[4]).

Four groups[5–8] reported full genomic screens using large numbers of microsatellite markers in MS families. All four studies confirmed the importance of the HLA gene complex

in 6p21, and, in addition, reported weak linkage to other loci. Interestingly, linkage to one of these loci was also suggested by Kuokkanen,[9] who used a candidate approach to 5p, guided by linkage to the syntenic locus in mouse to experimental autoimmune encephalomyelitis (EAE).[10]

Recently, a number of genomic regions regulating disease have been mapped in various experimental organ-specific inflammatory disease models. In the rat, these include insulin dependent diabetes mellitus (IDDM), autoimmune thyroid disease (ATD), experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA) and oil-induced arthritis (OIA).[11–17] In addition, susceptibility loci for specific autoimmune diseases appear to overlap with each other[18] suggesting that common genes or mechanisms are influencing different organ-specific inflammatory diseases.

Here, we have investigated the potential importance in MS of loci likely to be syntenic to chromosomal intervals that influence susceptibility to autoimmune disease in DA rats. Five quantitative trait loci (QTL) were originally identified by whole genome scans in CIA (Cia2-5) and OIA (Oia2).[16,17] We postulated that they might be of importance in autoimmunity or inflammation since DA rats are highly susceptible to many models of autoimmune diseases. Indeed, three of these loci were recently demonstrated to influence phenotypes of EAE induced by myelin oligodendrocyte glycoprotein (MOG) in DA rats (ie Cia3, Cia5 and Oia2).[13,19]

Twenty-seven microsatellite markers were investigated for linkage in 74 Swedish MS families. Markers in two chromosomal regions, 7q34-36 and 12p13-12, syntenic to Cia3 and Oia2, respectively, provided evidence for possible linkage. In addition, both were among those of possible importance according to previous genomic screens in MS.[5–8] Our observations indicate that genetic polymorphisms in these loci may influence immune regulation promoting autoimmune diseases regardless of target organ and species.

## Materials and methods
### Families and controls
All individuals were drawn from the relatively homogenous Swedish population. MS was diagnosed on the basis of clinical and paraclinical investigations.[20] Two hundred and eighty-six individuals were studied, including 132 patients. Types of families and numbers of patients are indicated in Table 1. Besides families, 148 ethnically matched healthy controls were used for determining allele frequencies. The study was approved by the local ethics committee.

### Genotyping
Genomic DNA was extracted from peripheral blood leucocytes using a modified salting-out protocol.[21] Polymerase chain reaction (PCR) was employed in 7 $\mu$l reaction volumes. Four hundred and thirty-three individuals were genotyped for 27 polymorphic markers corresponding to dinucleotide ($n$=17), trinucleotide ($n$=1), tetranucleotide ($n$=8) and penta-

nucleotide ($n$=1) repeats (Table 2). The Primers were synthesised by Scandinavian Gene Synthesis AB and forward primers for each primer pair were labelled with 5′- FAM, HEX, or TET phosphoramidites (Köping, Sweden). Optimal PCR conditions consisted of 95°C for 1 min, 55°C for 40 s and 72°C for 1 min with 30 cycles by a PCR cycle 9600 (Perkin Elmer), but may vary slightly among the markers. Genotyping was carried out using fluorescently labelled oligonucleotides in the semiautomatic GENESCAN/GENOTYPER system on an Applied BioSystems 377 DNA sequencing equipment, PCR products were diluted and 1.5 $\mu$l was combined with 2.5 $\mu$l internal lane size standard mixture (Genescan-350 or -500 TAMRA, Applied BioSystems). The accuracy and reproducibility of automated sizing of fragments was further confirmed by randomly chosen repeated analyses of identical samples and two readers independently reading data. Genotypes that were found to be inconsistent or which were discrepant between the two readers were either repeated or discarded.

### Synteny and markers
The identification of syntenic segments between rat and human chromosomal loci was achieved by comparing available mapping data for identified genes in human, mouse and rat genome database and homology map on the web sites (http://www.gdb.org, http://ratmap.gen.gu.se, http://www.informatics.jax.org, http://www.ncbi.nlm.nih.gov/Homology). The primer sequences of used markers were taken from human genome database (http://www.gdb.org). The rat Cia3 locus was found to correspond to four human chromosomal segments, in chromosomes 2p12, 3p25.3, 7q34-36 and 10q11.23. The Cia2, Cia4 and Cia5 loci were found likely to correspond to single human chromosomal regions, 19q13.1, 22q12-13 and 17q21.33-25.2 respectively. The Oia2 locus was found to correspond to a segment in chromosome 12p13-12. We selected a few markers corresponding to the various loci at first. Later on markers were added for two regions for further delineation.

### Statistical analysis
Since MS is considered a polygenic and possibly multifactorial disorder with an unknown mode of inheritance, non-parametric methods were used for linkage analysis. Linkage analysis was performed on data from multiplex families only. (a) The non-parametric linkage (NPL) analysis of the GENEHUNTER computer package.[22] The NPL scores are

**Table 1** Types of families and numbers of total individuals

| Affected/family | Familes (n) | Total individual (n) |
|---|---|---|
| 4 | 2 | 9 |
| 3 | 8 | 47 |
| 2 | 36 | 151 |
| 1 | 28 | 79 |
| Total | 74 | 286 |

**Table 2** Location, characteristics and outcome of linkage analysis in MS of 27 microsatellite markers syntenic to rat autoimmune disease loci

| Rat loci | Human homologous loci | Markers used | Distance to next marker (cM) | Genehunter NPL-score | APM P-value | ETDT P-value |
|---|---|---|---|---|---|---|
| Cia3 | 2p12 | CD8 | | −0.52 | 0.35 | 0.5 |
| | 3p25 | D3S1304 | 6.03 | 0.26 | 0.42 | 0.15 |
| | 3p25.3 | D3S2403 | | 0.69 | 0.03 | 0.32 |
| | 7q34 | D7S684 | 4.75 | −0.81 | 0.74 | 0.04 |
| | 7q35 | TCRβ/R-M | 0 | 0.49 | 0.0003 | 0.24 |
| | 7q35 | TCRβ/R-I | 0 | 0.62 | 0.06 | 0.22 |
| | 7q35 | TCRβ/R-G | 0 | 0.76 | 0.03 | 0.14 |
| | 7q35 | TCRVβ6.7 | 2.53 | 1.16 | 0.06 | 0.07 |
| | 7q36.1 | D7S2511 | 2.61 | 0.53 | 0.29 | 0.008 |
| | 7q36.1 | D7S1826 | | −0.16 | 0.89 | 0.06 |
| | 10q11.23 | D10S1426 | | −0.3 | 0.42 | 0.64 |
| Oia2 | 12p13.3 | D12S372 | 0.4 | 0.01 | 0.83 | 0.13 |
| | 12p13.3 | D12S93 | 0.75 | 0.92 | 0.0002 | 0.014 |
| | 12p13.3 | D12S356 | 0.37 | 0.61 | 0.016 | 0.29 |
| | 12p13.3 | D12S374 | 0.41 | 1.16 | 0.05 | 0.09 |
| | 12p13.3 | CD4 C/D | 0.06 | 0.12 | 0.89 | 0.39 |
| | 12p13.3 | D12S1625 | 2.77 | 0.36 | 0.02 | 0.009 |
| | 12p13.3 | D12S336 | 3.16 | −0.48 | 0.76 | 0.63 |
| | 12p13.3 | D12S391 | 6.96 | 0.21 | 0.17 | 0.09 |
| | 12p12.3 | D12S373 | 5.7 | 0.41 | 0.72 | 0.48 |
| | 12p12.1 | D12S1042 | | 0.01 | 0.85 | 0.03 |
| Cia5 | 17q21.33 | D17S1301 | 27.35 | −0.79 | 0.86 | 0.36 |
| | 17q25.2 | D17S784 | 2.33 | −1.52 | 0.94 | 0.07 |
| | 17q25.2 | D17S1830 | | −0.16 | 0.56 | 0.028 |
| Cia2 | 19q13.1 | D19S246 | | −0.46 | 0.28 | 0.31 |
| Cia4 | 22q12-13 | PDGFB1 | 0 | −0.24 | 0.84 | 0.44 |
| | 22q12-13 | PDGFB2 | | 0.49 | 0.07 | 0.24 |

model-independent and effectively measure the extent to which marker alleles shared by descent between affected individuals is greater than expected under random segregation. Initially, two-point linkage analysis was performed. In case the initial analysis was considered promising, arbitrarily defined as NPL > 1.0 or APM $P$ value <0.01, additional markers were added between and flanking the original markers and multipoint linkage NPL analysis was performed.

(b) APM (affected pedigree member, version 2.10).[23] Since this analysis is known to be sensitive to erroneous population marker allele frequencies, these were estimated from 148 normal controls.

(c) ETDT (extended transmission disequilibrium test, version 1.4),[24] which is a family-based linkage-disequilibrium test, was applied in transmission analysis of multiplex and singleton families together.

## Results
Results are summarised in Table 2.

### A region syntenic to Cia2: 19q13.1
The only selected marker in this locus failed to provide evidence for linkage (see Table 2). This fits well with our previously reported negative data concerning markers close

to the neighbouring myelin-associated glycoprotein (MAG) gene.[25]

### Four regions syntenic to Cia3: 2p12, 3p25, 7q34-36 and 10q11.23
For 2q12 and 10q11.23, we selected single marker, CD8 located intragenically in the CD8 gene and D10S1426 located in 10q11.23 to represent these two regions. As shown in Table 2, there was no evidence for an importance of these two loci. One of two markers selected for 3p25, however, showed slight evidence for linkage ($P$=0.03) by APM as well as a slightly positive NPL score of 0.69. However, there was no indication of importance as observed by TDT.

In the initial screen, we selected four intragenic markers in the TCRβ region in 7q34-36 at first, partly due to the previous interest in this locus. Slightly positive linkage was observed both by NPL and APM analysis (see Table 2). Therefore, three additional markers were added. Altogether, five out of seven markers showed slightly positive NPL scores, with a maximum score of 1.16 (marker TCRβ 6.7). Multipoint analysis did not increase this score, as the maximum multipoint statistic by NPL was 0.77. In order to avoid the negative side-effects of multipoint analysis with flanking markers and allow all meioses on all pedigrees to be scored in the analysis,[26] we also performed an analysis with only the five most tightly linked markers. However, this only increased the

multipoint NPL score marginally to 0.94. Significant linkage was observed by APM ($P$=0.0003 for marker TCR $\beta$/R-M) and TDT ($P$=0.008 for marker D7S511).

### A region syntenic to Cia4: 22q12-13
For two selected markers in this region, no evidence for linkage was observed.

### A region syntenic to Cia5: 17q21-25
We investigated three markers in 17q21-25. Apart from a marginal transmission distortion for marker D17S1830, no evidence for linkage was observed.

### A region syntenic to Oia2: 12p13-12
This locus includes the CD4 gene, a highly interesting candidate gene in MS. The observation of possible linkage in the neighbouring two markers (see Table 2) prompted us to add more flanking markers to obtain efficient delineation of the locus. Overall, the strongest evidence for linkage was observed for marker D12S374 by NPL analysis (1.16), and for marker D12S93 by APM ($P$=0.00002). Here, three adjacent markers in a 1.1 cM region scored positively by both NPL and APM analyses. As for the TCR$\beta$ locus, multipoint analysis did not increase the NPL score, which fell slightly to 1.01. On the other hand, a restricted analysis of only the most informative families (those containing affected sibling pairs) increased the NPL slightly to a maximum of 1.47.

## Discussion
We have observed possible evidence for linkage in MS for two loci, 7q34-35 and 12p13-12 in the comparatively homogeneous Swedish population. These loci were identified due to synteny with loci of clear importance for autoimmune model diseases in the rat, and also overlap with areas showing promising linkage in previous MS genomic screens (see Table 3).[5,6] Furthermore, a similar approach has been shown to be successful concerning another strong candidate locus in MS, 5p,[9] an area syntenic to a locus of importance for mouse EAE.[10] In fact, also for this locus we have in the present material previously reported possible linkage as well as association.[27]

One of the identified loci, 7q34-36, contains the TCR$\beta$ gene cluster. The TCR$\beta$ gene has indeed been intensively investigated as a candidate susceptibility gene in MS. Thus, indications of linkage have been reported[6,28,29] but also failed to be observed.[5,7,30] Likewise, some studies have shown association[31,32] whereas others have not.[33,34] These inconsistent findings have been obtained both by simple biallelic markers as well as by polyallelic microsatellite markers. In addition, in two frequently used mouse models of MS, EAE and Theiler's murine encephalomyelitis virus (TMEV) infection, the TCR$\beta$ chain locus has been reported to be a major susceptibility gene if co-inherited with the major histocompatibility complex (MHC).[13,35,36] In fact, several studies have indicated that the Cia3 region contains a gene that controls

the development of several autoimmune disease-related traits.[11,37,38]

There are, naturally, a number of possible explanations for the discrepancy among studies on complex disorders such as MS. Locus heterogeneity may be present, where the absolute or relative importance of different loci vary between populations due to the interaction of genes or a different distribution of alleles. Further, the low relative risk of each genetic factor in polygenic disorders and a limited sample size easily lead to lack of adequate power for replication of previous observations. Finally, it may still be that some results are the effect of random variation and do not reflect the presence of a susceptibility locus. Anyhow, there are at present several promising observations concerning the TCR$\beta$ region in MS, hopefully inspiring further efforts.

The other most interesting locus in the present study was 12p13-12, being syntenic to the rat Oia2 locus. Previously, this region has been suggested to be of importance in the British genomic screen study.[5] Intriguingly, the CD4 antigen has a prominent role in potentiating antigen recognition by helper T cells. Brown[39] reported that CD4-deficient, T cell receptor transgenic T cells were incapable of Th2 differentiation after *in vitro* activation. Expression of a wild-type CD4 transgene corrected the Th2 defect of CD4-deficient mice in all immune responses tested. Interestingly, this region has substantial overlap also with loci of importance in myelin-oligodendrocyte glycoprotein- (MOG)-induced EAE,[13] experimental diabetes,[40] lupus[41] and B. Pertussis-induced histamine sensitisation.[42]

As in a previous report,[43] we arbitrarily used NPL > 1 and APM $P$ < 0.01 to define a region as promising, well aware that this definition falls short of suggested criteria.[44] Thus Landers and Kruglyak suggested a $P$ value of < 0.002 to define a region 'suggestive'. Although we did observe such $P$ values for both main loci, most of our findings were less convincing. In addition, we have analysed our data for multiple markers with multiple methods and are aware that this may increase the risk of false positive results. We believe, however, that our conclusions are supported in three ways. First, the fact that several adjacent markers show similar results indicate that in the present material there is, in fact, a weak but true co-segregation of disease in these particular regions. Second, these exact regions were not only supported by previous findings in animal models but were also previously identified as interesting regions in MS as stated in Table 3. Third, in a key study, Becker *et al* pointed out the clustering of non-MHC loci in supposedly autoimmune polygenic disorders and several experimental model diseases.[18] The present data add further support to this notion, as both 12p13-12 and 7q34-36 are among the loci pointed out by Becker. One interpretation of this pattern is, of course, that these loci harbour genes that are key regulators of pathogenic immune responses. Such genes would regulate autoimmune disease in a target organ-independent fashion and may be considered as 'autogenes'.[45]

**Table 3** Summary of studied candidate chromosomal regions compared with previous human studies as well as studies in experimental autoimmune model diseases. Please note that, for human studies, suggestive evidence for linkage is indicated as positive, although falling short of, for instance, genome-wide significance

| Human locus | Present study | | | Autoimmune models in rodents | | | | | Published genomic screens | | | |
| | NPL >1.0 | TDT P<0.05 | APM P<0.01 | Rat locus (chr) | EA | EAE | IDDM | ATD | US | UK | Canada | Finland |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3p25 | − | − | − | Cia3(4) | + | + | + | + | − | − | + | − |
| 7q34-36 | + | + | + | Cia3(4) | + | + | + | + | + | − | − | − |
| 12p13-12 | + | + | + | Oia2(4) | + | + | | | − | + | − | − |
| 17q22-25 | − | − | − | Cia5(10) | + | + | | | − | + | − | − |
| 19q13 | − | − | − | Cia2(1) | + | − | | | + | + | + | ± |
| 22q13 | − | − | − | Cia4(7) | + | − | | | − | + | − | − |

NPL, non-parametric linkage analysis; TDT, transmission disequilibrium test; APM, affected pedigree member analysis; EA, experimental arthritis; EAE, experimental allergic encephalomyelitis; IDDM, insulin dependent diabetes mellitus; ATD, autoimmune thyroid disease.

We are especially encouraged by the apparent significance in MS of genomic regions defined in experimental inflammatory diseases where prospects for positional cloning of genes are promising. After exact positioning in rats or mice, human susceptibility genes may be readily identified.

The initial response to the publication of the three genomic screens in MS in 1996[5–7] was one of disappointment,[46] since data supporting non-HLA loci seemed weak. However, subsequent data[8,27,47] including the present study show a surprising consistence. Thus, based on available data, the following loci are likely to contain MS susceptibility genes having shown evidence for linkage: 6p21 (HLA complex), 5p, 17q21-25, 7pter-15, 7q34-36, 12p13-12, and 12q23. It now seems reasonable to focus on identifying the responsible genes, particularly as this may be more rewarding than previously recognised due to the possible relevance in other autoimmune disorders.

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