LETTERS TO THE EDITOR

No evidence for the involvement of interleukin 2 or the immunoglobulin heavy chain gene cluster in determining genetic susceptibility to multiple sclerosis

Here we report the investigation of two promising candidate multiple sclerosis susceptibility genes. Each is biologically plausible, having a function suggesting possible involvement in the pathogenesis of the disease and positional, having existing linkage evidence supporting its candidature. The two differ, however, in the origin of the supporting linkage evidence. This comes mainly from the analysis of animal models in the case of interleukin 2 (IL-2)[1] and from human studies in the case of the immunoglobulin heavy chain gene cluster.[2]

Interleukin 2 is a cytokine intimately involved with both the function and regulation of the immune system. It has both proinflammatory and anti-inflammatory actions, promoting T cell proliferation during cell mediated immune responses and, conversely, being crucial both for the development and maintenance of self tolerance. Genetic analysis of experimental autoimmune encephalomyelitis (EAE) provides strong evidence supporting the candidacy of IL-2 as a susceptibility gene.[1]

The immunoglobulin heavy chain gene cluster is another highly promising candidate. Plasma cells and B lymphocytes are readily detected in areas of acute demyelination and the occurrence of oligoclonal immunoglobulin bands in the spinal fluid of affected people is a distinctive feature of the disease. Moreover, the cluster is encoded towards the telomere of chromosome 14q where linkage evidence from the United Kingdom sibling pair families is at its strongest (lod score≈3.0).[2]

The gene for IL-2 is encoded on chromosome 4q26. To investigate its role as a susceptibility factor in multiple sclerosis, we typed a closely encoded microsatellite marker in 502 trio families (both parents and a single affected offspring). Transmission disequilibrium testing (TDT)[3] of these data disclosed no significant evidence for linkage disequilibrium (table). The expression of IL-2 is under the control of transcription factor 8 (TCF8), the gene for which is encoded on chromosome 10p11.[4] Because variation in IL-2 expression could contribute to susceptibility of multiple sclerosis, we also typed a microsatellite encoded close to the TCF8 gene in the same 502 families. Again, the TDT results (table) were negative.

We typed three microsatellite markers encoded within the immunoglobulin heavy chain gene cluster in 460 simplex families. Once again TDT failed to show evidence for linkage disequilibrium (table) at any of these markers. As the markers are encoded within a 200 kb region, we also subjected them to multipoint TDT analysis but no haplotypes showing significant transmission distortion were found.

These results suggest that neither of the tested candidates has any major effect in determining genetic susceptibility to multiple sclerosis. However, in considering these data it is important to remember that the negative results could represent a type II error as, even with the large numbers of simplex families used, the power of this type of candidate gene study is limited when the effects attributable to the susceptibility genes are modest. A further possibility is that the available evidence for linkage is falsely positive and that, in fact, no susceptibility genes are encoded in these regions. The lod score observed in the immunoglobulin heavy chain gene cluster region is significantly short of the 5% genomewide significance threshold suggested by Lander and Kruglyak (lod score=4.0).[5] A third possibility is that the linkages are genuine but unrelated to the candidates we have tested. We favour this explanation with the available data suggesting that alternative candidates from these regions are responsible for the observed linkages.

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ROBERT FEAKES
STEPHEN SAWCER
BELINDA SMILLIE
JEREMY CHATAWAY
SIMON BROADLEY
ALASTAIR COMPSTON
University of Cambridge Neurology unit, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, UK

DAVID CLAYTON
MRC Biostatistics Unit, Institute of Public Health, University Forvie Site, Robinson Way, Cambridge, CB2 2SR, UK

ALASTAIR COMPSTON
ED Adrian Building, University Forvie Site, Robinson Way, Cambridge, CB2 2SR, UK

Correspondence to: Professor Alastair Compston
alastair.compston@medschl.cam.ac.uk

Table 1 Transmission disequilibrium testing results

Marker	*Het*	χ^2	*df*	*p Value*	*Primers*
IL2	0.89	7.31	4	0.12	AAA GAG ACC TGC TAA CAC TGT TTC CCC TTG CCG CC
TCF8	0.73	0.08	2	0.96	AGA GGA TCC TGT TCA CTA CTG TGC GAA TTC TTA CTA GGT CTG AGG
D14S1419	0.56	2.31	3	0.51	TAG GGA CAG GCA GTT GAT TA CAA TTA ATG TAA AAA TTA GCC A
D14S1420	0.67	0.74	2	0.69	TGT TTG AAG AAG GGA GTC GT CCC ACT CCA TGT CTT CTG TT
D14S826	0.74	1.74	4	0.78	TCT CTA AAG CTA CTA TAA CCC AG TGC TGT TGG ACT CAG GTA GCT A

Each microsatellite was amplified by PCR from genomic DNA with fluorescent labelling of the forward primer and genotyped using the Applied Biosystems GENESCAN/GENOTYPER system (primers as shown in table). TDT was performed using the TRANSMIT program version 2.5, considering only those alleles with a frequency of greater than 10% (corresponding to the number of degrees of freedom {df} in the table). The chromosome 14 markers are listed in map order.
The families were recruited from throughout the United Kingdom. All are white and the affected offspring meet the Poser criteria, 95% having clinically definite, category A or B, disease.

1 Encinas JA, Wicker LS, Peterson LB, *et al*. QTL influencing autoimmune diabetes and encephalomyelitis map to a 0.15-cM region containing IL2. *Nat Genet* 1999;**21**:158–160.
2 Feakes R, Chataway J, Sawcer S, *et al*. Susceptibility to multiple sclerosis and the immunoglobulin heavy chain gene cluster. *Ann Neurol* 1998;**44**:984.
3 Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 1996;**59**: 983–9.
4 Williams TM, Moolten D, Burlein J, *et al*. Identification of a zinc finger protein that inhibits IL-2 gene expression. *Science* 1991;**254**:1791–4.
5 Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995;**11**: 241–7.

Coma in a patient with Alzheimer's disease taking low dose trazodone and ginkgo biloba

We describe a patient with Alzheimer's disease who developed coma a few days after starting low dose trazodone associated with ginkgo biloba. Coma was reversed by flumazenil, a specific antagonist of the benzodiazepine (BDZ) receptor. The finding is relevant in that, although the sedative effects of trazodone are well known, the drug is inactive on the BDZ receptor. On the other hand, ginkgo is active on the receptor, but sedation has so far never been reported.

In March 1999, an 80 year old woman was first evaluated in our facility and given a diagnosis of probable Alzheimer's disease (NINCDS-ADRDA criteria) of a moderate severity (mini mental state examination of 10/30). She had no physical comorbidity or vascular risk factors. At the time of observation she was taking 3.5 mg bromazepam for mild restlessness, anxiety, and irritability, with only partial benefit (neuropsychiatric inventory: anxiety 4/12, irritability/lability 3/12). A dose of 5 mg donepezil at bedtime was added with the aim of improving both cognitive function and behaviour, together with 600 mg vitamin E twice daily.

After 3 months, no improvement of cognitive function, behaviour, or daily function could be detected. Donepezil was discontinued. Vitamin E was also discontinued due to the development of ecchimotic bruises on all limbs. Ginkgo biloba ((Egb 761) 80 mg) twice daily was added. Rivastigmine was not considered a feasible option because it was not possible to have frequent clinical follow up visits during the titration phase. For a better control of behavioural disturbances, bromazepam was replaced with 20 mg trazodone twice daily.

The day after the visit, the new therapeutical regimen was initiated. Sedation or other adverse effects did not appear, and the care giver reported improvement of anxiety. On the next day, the improvement of behavioural disturbances was sustained, still in the absence of sedation. At 600 pm of the third day, the patient developed instability of gait and drowsiness. At 700 pm she fell asleep. The care giver tried to wake her by slapping her face, but without success. Overall, she had taken 100 mg trazodone and 320 mg Egb 761 in about 50 hours. A physician on call found that blood pressure was 120/55 mm Hg and her Glasgow coma scale was 6/15. The patient was taken to the nearest hospital,