

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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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.

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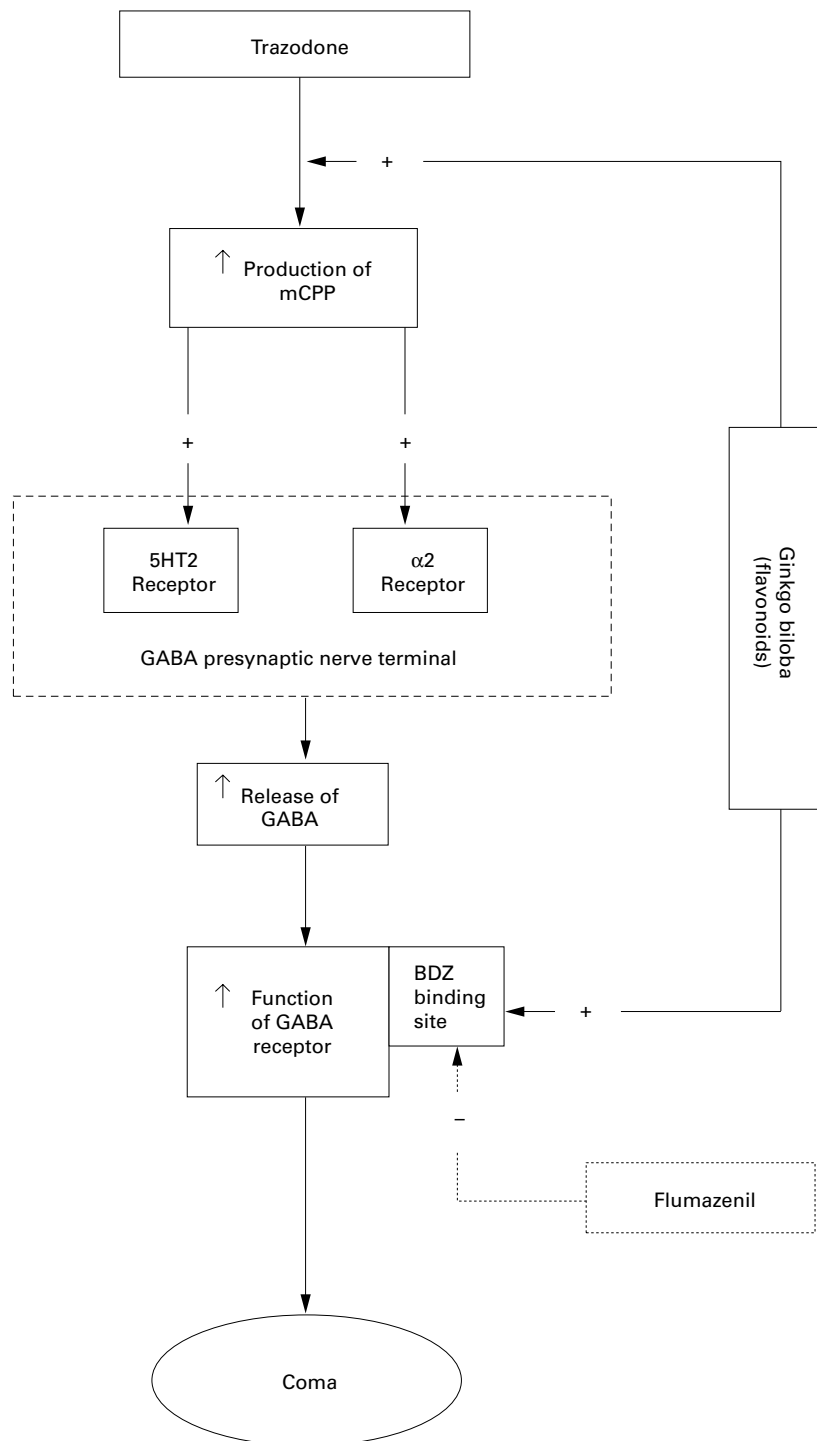
Coma in a patient with Alzheimer's disease taking low dose trazodone and ginkgo biloba

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/labidity 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 ecchymotic 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 therapeutic 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,



Pathway from trazodone to coma.

where 1 mg flumazenil intravenously was administered. The patient awoke immediately.

Medicine bottles at the patient's home were examined. The medications remaining in the containers were consistent with the prescribed doses, and medications other than those prescribed were not found anywhere in the house.

Trazodone and ginkgo were discontinued and bromazepam was resumed. The patient was re-evaluated 2 months later. Cognitive functions and behaviour were unchanged.

The pathogenesis of coma would have remained unexplained in this patient had not

flumazenil been fortuitously administered. This led to an investigation of the links among trazodone, ginkgo, and the GABAergic system.

Ginkgo biloba is active on cognition in Alzheimer's disease through its antioxidant properties. Flavonoids represent the major active component of the extract and possess GABAergic activity through action at the BDZ binding site as partial agonists.¹ However, ginkgo alone does not have clinically sedative effects.

Trazodone is an antidepressant widely used for its hypnotic and sedative effects to treat behavioural disturbances in the de-

mented. Drowsiness is a dose dependent effect due to a direct antagonism at the α_1 adrenergic receptor level, and in elderly people it has been reported with doses greater than 75 mg daily. Trazodone is metabolised by an isoform of cytochrome P-450 (CYP3A4) into an active compound 1-(m-chlorophenyl)piperazine (mCPP). Through an agonistic action on presynaptic 5-HT₂ and α_2 receptors located on GABAergic nerve terminals,² mCPP enhances the release of GABA.^{3,4} The activity of CYP3A4 can be increased by flavonoids.⁵

We hypothesise that, in our patient, on the one hand flavonoids provided a subclinical increase of the GABAergic activity through a direct effect on the BDZ receptor, whereas on the other increased CYP3A4 function and mCPP production, inducing a further enhancement of GABAergic activity that became clinically apparent. Flumazenil might have blocked the direct effect of flavonoids, thus causing GABAergic activity to fall below the threshold of clinical manifestation (figure).

Ginkgo is widely used and is thought to be a harmless food supplement. Although we warn that the adverse effects of the simultaneous administration of trazodone and ginkgo should be further confirmed, we think that natural compounds such as ginkgo can have, in some clinical circumstances, adverse effects of similar magnitude as traditional drugs.

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Bickerstaff's brainstem encephalitis subsequent to *Campylobacter jejuni* enteritis

Bickerstaff's brainstem encephalitis (BBE) is characterised by acute ophthalmoplegia and ataxia preceded by infection.¹ BBE rather than Miller Fisher syndrome is usually diagnosed for patients who show drowsiness and have brisk reflexes, extensor plantar responses, and long tract sensory disturbance. Bickerstaff and Cloake¹ speculated that the aetiology of BBE is similar to that of