LETTERS

Crohn's associated NOD2 gene variants are not involved in determining susceptibility to multiple sclerosis

Autoimmune diseases, such as multiple sclerosis and Crohn's disease, are believed to result from the effects of environmental agents acting on genetically susceptible individuals. Evidence from segregation analysis and systematic whole genome linkage studies indicates that the nature of this susceptibility is complex, involving several genes which each individually confer only modest excess risk. Recurrence risk analysis in the relatives of affected individuals[1] together with the comparison of whole genome linkage studies across these diseases[2] shows that there are likely to be both genes conferring an autoimmune diathesis in general and others determining precisely which autoimmune phenotype may result. On this basis it is reasonable to hypothesise that genes shown to be relevant in one autoimmune disease may be of importance in another and therefore offer themselves as potential candidates.

During the last few years striking progress has been made in unravelling the genetic basis of susceptibility to Crohn's disease. Significant evidence for linkage in the pericentromeric region of chromosome 16 has been found,[3] following on from which two independent groups, one using association mapping[4] and the other following a candidate gene approach,[5] identified the relevant gene as NOD2. Three variants of this gene (IBD8, IBD12, and IBD13) were shown to influence susceptibility to Crohn's disease. IBD8 is a missense mutation in exon 3 (2023C>T, R675W); IBD12 is a missense mutation in exon 7 (2641G>C, G1881R); and IBD13 is a frameshift variant in exon 10 (2936insC, 980fs981X). Although precise functions of the NOD2 gene are not fully known it is believed to have important immunological activity, particularly in maintaining symbiosis between the gut lining and its commensal bacteria.

Given the established importance of these variants in determining susceptibility to one autoimmune disease (Crohn's disease), we examined their role in a second by genotyping all three variants in a large number of patients with multiple sclerosis (n = 631) and a cohort of controls (n = 343).

All individuals taking part in this study gave informed written consent for genetic analysis. Each individual gave a venous blood sample from which DNA was extracted using standard methods. Genotyping was undertaken using Applied Biosystems multiplex primer extension assay system (Multiplex SNaPshot). Primers for primary PCR amplification and extension reactions are shown in table 1. Electrophoresis was done on a 3700 DNA analyser with genotyping completed using the GENSCAN/GENOTYPER software systems. Statistical analysis was by χ^2 testing.

The observed allele frequencies are shown in table 1. No statistically significant difference in allele frequency was seen for IBD8 (χ^2 = 1.57, p = 0.21), IBD12 (χ^2 = 0.002, p = 0.96), or IBD13 (χ^2 = 2.78, p = 0.10). In each case, the observed allele frequency was commensurate with that previously observed in the Crohn's disease studies (table 1).

Our results indicate that the NOD2 gene is probably not influencing susceptibility to autoimmune disease in general but is specific for Crohn's disease.

Table 1 Observed frequency of Crohn's disease associated alleles in multiple sclerosis

Variant	Multiple sclerosis (%)	Controls (%)	Published control frequency (%)
IBD8*	54 (4.8)	34 (6.2)	4
IBD12	11 (0.9)	6 (0.9)	1
IBD13	28 (2.3)	8 (1.2)	2

*The primary PCR for this assay was relatively unreliable such that typing success rate was 90% for cases and 80% for controls. Both of the other assays had typing success rates of greater than 95%. The manufacturer's standard reaction conditions were used for all reactions except the primary amplification of IBD8 where a lower annealing temperature of 50°C was used along with four additional PCR cycles.
Primary PCR primers
IBD8: ACCTTCAGATCACAGCAGCC and GCTCCCCCATACCTGAAC
IBD12: AAGTCTGTAATGTAAAGCCA and CCCAGCTCCTCCCTCTTC
IBD13: CTCACCATTGTATCTTCTTTTCC and GAATGTCAGAATCAGAAGGG
Extension primers
IBD8: TTTTTTTTTTTTTCATCTGAGAAGGCCCTGCTC
IBD12: TGGCCTTTTCAGATTCTGG
IBD13: TTTTTTTGGTGTCATTCCTTTCAAGGG

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Favourable outcome of a brain trauma patient despite bilateral loss of cortical somatosensory evoked potential during thiopental sedation

We would like to present an observation that somewhat questions the predictive value of somatosensory evoked potentials on the outcome of brain trauma patients treated with thiopental coma.[1][2]

A 30 year old woman suffered a high velocity car accident resulting in a diffuse brain injury. Her Glasgow coma scale score on admission was $E_2V_2M_5$ (9/15), with preserved pupillary reflexes and gross motor function. Computed tomography of the head showed a traumatic disjunction of the lambdoid suture and multiple left frontobasal and temporal cerebral contusions. The patient was sedated with propofol, intubated, and monitored for intracerebral pressure (ICP) through an external ventricular drain. Her clinical condition rapidly worsened because of brain swelling around the contusions, and cerebrospinal fluid drainage, manitol boluses, and mild hyperventilation were started. Three days after admission, a further ICP increase was treated with thiopental coma (10 mg/kg/h × 24 h loading dose followed by 3 mg/kg/h maintenance dose to obtain a burst suppression EEG pattern). On day 7, the patient developed a left sided mydriasis and a left temporal partial lobectomy was performed to remove contused brain. The ICP returned to normal and thiopental administration was stopped on day 8. On day 10, the EEG was isoelectrical and on day 11, somatosensory evoked potentials (SSEP) of the median nerve showed no cortical response (N20) despite normal brachial plexus (Erb) and lemniscal (P14) potentials. Levels of thiopental and phenobarbital, its main metabolite, were then respectively 65 ng/l and 56 ng/l. The patient remained areactive (GCS 3/15$_T$) and without brain stem reflexes, including the ocular-cardiac response, until day 20. The transcranial Doppler however showed normal flow patterns and the brain CT scan did not reveal any post-herniation ischaemic lesion. On day 21, the patient opened her eyes. The serum concentration of thiopental was then 12 ng/l whereas that of phenobarbital remained around 40 ng/l until day 23. A 1–2 Hz low amplitude EEG activity with right sided predominance was observed, and the SSEP cortical peak N20 recovered on day 22 when the thiopental concentration was 5.9 ng/l. A steady improvement followed. On discharge to a rehabilitation facility (day 57), the patient could follow simple commands but suffered mixed dysphasia and generalised weakness. At four months, she presented no residual motor deficit, an improved verbal expression and comprehension, and a moderate frontal behaviour. At two years, the patient only still suffered some episodes of labile mood, and although she had not resumed her previous job, she was active as a farm worker, read and wrote, drove her car, and could live an independent and social life, with a Glasgow outcome score (GOS) of 5/5.

SSEP are commonly used to monitor comatose patients even under barbiturate sedation.[2][3] Indeed, although their morphology can become changed, short latency SSEPs

in humans supposedly do not disappear in response to barbiturate doses sufficient to render the EEG isoelectric and the neurological examination similar to brain stem death.[3][4] The bilateral loss of SSEP N20 responses is regarded as a predictor of ominous outcome after a trauma. There are only a few reports on the recovery of initially absent or lost N20 potentials after severe brain injury with increased ICP, some of them with a good outcome as was the case in our patient.[5][6] In our case, the disappearance of the cortical evoked responses correlated with both the ICP increase and the induction of thiopental coma. As their reappearance closely matched the elimination of thiopental from the bloodstream and was quite delayed relative to the normalisation of the ICP, our observation suggests that barbiturates may contribute to the suppression of N20 evoked potentials in brain trauma patients. Awaiting further observations, caution is thus warranted on the use of SSEP to monitor the clinical evolution and predict the outcome of such patients under barbiturate coma.

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Epidemiology of the mitochondrial DNA 8344A>G mutation for the myoclonus epilepsy and ragged red fibres (MERRF) syndrome

The myoclonus epilepsy and ragged red fibres (MERRF) syndrome is a maternally inherited progressive mitochondrial encephalomyopathy caused by a 8344A>G mutation in the MTTK gene that encodes mitochondrial tRNA for lysine. Its common clinical features include myoclonic and tonic-clonic seizures, ataxia, and myopathy, but other features have also been reported, including lipoma, diabetes mellitus, optic atrophy, peripheral neuropathy, hearing loss, and dementia.[1]

The population frequencies of pathogenic mutations in mitochondrial DNA (mtDNA) are not well known, but the Finnish healthcare organisation provides good opportunities to carry out studies on molecular epidemiology. We have previously determined the frequency of 3243A>G, the most common cause of the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), to be 16/100 000 in the adult population of Northern Ostrobothnia.[2] We report here on the identification of patient groups with common clinical features of the MERRF syndrome, in a comparable population and the resulting determination of the prevalence of the 8433A>G mtDNA mutation.

Patients and methods

The prevalence area considered here is the province of Northern Ostrobothnia in northern Finland, with a total population of 353 895 on 31 December 1994 (prevalence date), including 245 201 persons ≥20 years of age. Adult patients with diagnoses that are commonly associated with the 8344A>G mutation[1] were identified as being at risk with respect to mitochondrial disorders, and we therefore screened the population for patients ≥20 years of age who had disorders such as ataxia, diabetes mellitus, epilepsy, lipoma, myopathy, ophthalmoplegia, optic atrophy, peripheral neuropathy, and sensorineural hearing impairment (table 1). These were

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Table 1 Criteria used in the screening of the patient groups

Patient group	Selection criterion 1	Number of patients identified	Selection criterion 2	Number of patients identified	Number (%) of samples received
Ataxia	Any ataxia, unknown aetiology	79	Idiopathic cerebellar ataxia, age ≥20 years at visit	39	26 (67)
Diabetes*	Insulin treatment started at age 20–45 years	479	Family history of mitochondrial phenotype†	169	143 (85)
Epilepsy‡	Age ≥20 years at visit, response to family history questionnaire	945	Family history of mitochondrial phenotype†	223	165 (74)
Hearing loss§	Sensorineural hearing impairment, hearing aid obtained at age ≤45 years, current age ≥20 years	242	Family history of mitochondrial phenotype†	108	82 (76)
Lipoma	Any lipoma	621	Axial or multiple lipomas, age ≥20 years at visit	150	107 (71)
Myopathy	Any myopathy with clinical and EMG verification, age ≥20 years at visit	146	Myopathy of unknown aetiology or any muscle dystrophy¶	41	32 (78)
Neuropathy	Any electrophysiologically defined idiopathic neuropathy, age ≥20 years at visit	138	Familial neuropathy or family history of mitochondrial phenotype†	31	21 (68)
Ophthalmoplegia	Double vision or ptosis, any age	799	Definite ophthalmoplegia or symmetric ptosis, age ≥20 years at examination	15	15 (100)
Optic atrophy	Decrease in visual acuity or optic disc abnormality, any cause, any age	1542	Optic atrophy of unknown aetiology**, current age ≥20 years	42	30 (71)
	Total	4991	Total	818	621 (76)

OUH; Oulu University Hospital. Computer search at OUH was first performed to identify patients with specific discharge diagnoses that had been filed according to Finnish version of the International Statistical Classification of Diseases and Related Health Problems. Specific selection criteria were then applied to select patients with definite diagnoses. *Patients with insulin dependent diabetes mellitus obtain needles, syringes, insulin pens, and glucose sticks free of charge from the public health care units, and the supplies used are recorded. These patients were identified from the records of 40 of the 42 local authority health care units. Discharge diagnoses at one of the two regional hospitals in the area and the diabetes register of the other also were reviewed. †Patients with any combination of diabetes mellitus, sensorineural hearing impairment or epilepsy in first or second degree maternal relatives were included. ‡Most adult patients with epilepsy make regular follow up visits to the outpatient clinic of the department of neurology at OUH at least once a year. During a one year period, a physician involved in the study checked the charts of the patients visiting the clinic every day. The diagnosis of epilepsy was confirmed on this occasion, and patients receiving regular antiepileptic medication were included. No distinction was made between the types or aetiologies of epilepsy. §The cost of hearing aids is refunded in full by the public health service, and aids are supplied in the region only by the department of otorhinolaryngology at OUH. The register of hearing aids was reviewed and patients were ascertained on the basis of the following clinical criteria: symmetric sensorineural hearing impairment with undefined aetiology; hearing impairment >30 dB (pure tone average of frequencies 0.5, 1, 2, and 4 kHz); a difference between the ears <10 dB; and use of a hearing aid at age <45 years. ¶Duchenne muscular dystrophy and other myopathies with definite molecular genetic diagnosis were excluded. **Demyelinating diseases and ischaemic diseases were excluded.

ascertained as described in detail previously.[2] The research protocol was approved by the ethics committee of the Medical Faculty of the University of Oulu, Finland, and the Finnish Ministry of Social Affairs and Health.

DNA from blood samples was purified using the QIAamp Blood Kit (QIAGEN) and a fragment encompassing the MTTK gene was amplified by PCR in the presence of ^{35}S-dATP. The 8344A>G mutation was detected by restriction fragment analysis using *BglI*. After digestion, the samples were electrophoresed through a 6% acrylamide gel, which was dried and autoradiographed at −72°C overnight using Kodak XAR-5 film with an intensifying screen. Amplified DNA from a subject known to harbour the mutation was included in each restriction digestion and electrophoresis. The degree of mutant heteroplasmy in this sample was 59%.

Results and Comment

We identified 818 patients with signs or symptoms that have been associated with MERRF (table 1), and samples obtained from 621 of these were examined for the 8344A>G mutation. None of the patients harboured the mutation (95% confidence intervals (CI) 0 to 3.67). The prevalence of 8344A>G in the adult population of Northern Ostrobothnia was thus calculated to be 0–1.5/100 000. The estimated frequency of 8344A>G in northern Finland is much lower than that of 3243A>G, but comparable to that found in two previous studies: 0.25/100 000 (95% CI 0.01 to 0.50) among adult patients in a single neurology centre in United Kingdom over a 10 year period,[3] and 0 to 0.25/100 000 (95% CI) in a population based study among children in western Sweden.[4] The 8344A>G mutation is not absent in Sweden or Finland, however, as the authors are aware of two families in southern Finland who possess it, and a few such families have been reported in Sweden.[4]

The frequency of 3243A>G has been found to be four times that of 8344A>G in the United Kingdom.[3] Furthermore, gene analyses in a molecular diagnostic laboratory have revealed that the ratio of these two mutations among 2000 patients with features of mitochondrial disorders is 4,[5] suggesting that the frequency ratio between the two is fairly constant. The 3243A>G MELAS mutation appears to be clearly more common than 8344A>G also among Finnish patients that was ascertained in a population based manner.

MtDNA mutations are a comparatively common cause of neurometabolic disorders in both adults and children, but they vary in prevalence. The most common mtDNA point mutations seem to be 11778G>A, 3243A>G and 3460G>A, while 8344A>G is infrequent. The 3243A>G mutation has arisen several times in a population[2] and is not faced with any strong selection pressure,[6] but the low frequency of 8344A>G suggests either that this gene is not a hot spot for mutational events, or that the mutation is rapidly eliminated in a population. Indeed, the two mutations lead to different biochemical consequences at the cellular level. The MERRF mutation impairs mitochondrial translation more severely than does the MELAS mutation.[7] Evolutionarily, these two mutations may therefore be faced with different negative selection and may explain the differences in population frequencies.

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Shifts in angiotensin I converting enzyme insertion allele frequency across Europe: implications for Alzheimer's disease risk

Early studies suggested that angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (ACE) gene polymorphism is associated with an increased risk of coronary artery disease and, more recently, with sporadic late onset Alzheimer's disease.[1] Studies conducted in northern European populations have considered the ACE*I allele to be a risk factor for various types of cognitive decline.[1 2] One such study in a French population found an association between the ACE*D allele and dementia,[3] while other studies in southern European populations found either a slight but significantly increased frequency of ACE*I in Alzheimer's disease patients[4] or did not detect any effect of ACE polymorphism.[5]

Our group recently reported the novel finding that apolipoprotein E (APOE) ε4 allele shows a geographical trend, decreasing in frequency from northern to southern Europe.[6] We hypothesised that the variability in the strength of evidence for an association between ACE polymorphism and Alzheimer's disease was related to similar geographical variations in ACE*I frequency. We investigated whether there was evidence in southern Italy of an association between the ACE polymorphism and increased risk of Alzheimer's disease. Secondly, we compared our results with the findings from published studies on other European populations.[1 2 4]

Between June 1998 and October 2001, we consecutively examined in our centre 141 patients with Alzheimer's disease (51 men, 90 women; mean (SD) age at onset, 71 (8.5) years), and 268 unrelated caregivers, spouses, friends, neighbours, or volunteers (118 men, 150 women; mean age at collection, 72 (7.1) years). A clinical diagnosis of probable Alzheimer's disease was made according to the criteria of the National Institute for Neurological and Communicative Disorders and Stroke/ Alzheimer's Disease and Related Disorders Association, and the group of non-demented elderly control subjects was sex and age matched. The ascertainment, diagnosis, and collection of cases and controls are described in detail elsewhere.[6] The age at onset of Alzheimer's disease symptoms was estimated from semistructured interviews with the patients' caregivers. The study protocol was approved by the ethics committee of the University of Bari. After a complete explanation of the study, written informed consent was obtained from all the subjects or their relatives.

APOE genotypes were determined as detailed elsewhere.[6] ACE genotypes were produced using established methods, followed by a quality control amplification step necessary in detecting underamplified ACE*I alleles.[1]

The statistical analysis was performed by Pearson χ^2 test to make genotype and allele comparisons as well as test for agreement of data with Hardy-Weinberg principles. Allele frequencies were determined by allele counting. To express variances of the allele frequencies, we used 95% CIs, calculated by Wilson's formulas. The differences among age at onset of Alzheimer's disease symptoms in relation to different ACE genotypes were calculated with Mann-Whitney test. To evaluate whether the association between Alzheimer's disease and ACE genotypes were homogeneous in all APOE strata we used a permutation based exact logistic model by LogXact procedure implemented in the SAS system (Proc-LogXact 4; Copyright 2001 by CYTEL Software Corporation, Cambridge, MA 021139). In order to correct for multiple statistical testing, the results were adjusted according to Bonferroni inequality. The Cochran-Armitage trend test was carried out to evaluate the geographical trend among ACE allele and genotype frequencies in Alzheimer's disease patients and controls of three European countries (Italy, Spain, and United Kingdom), from published studies.[1 2 4] The data were analysed by SAS FREQ procedure (version 8.2).

Table 1 shows ACE allele and genotypes frequencies in Alzheimer's disease patients and controls in southern Italy. The frequencies of the different ACE genotypes in our population were in Hardy–Weinberg equilibrium (HWE) (cases: Pearson χ^2 = 2.09, p = 0.15; controls: χ^2 = 2.49, p = 0.11). Moreover, there was no

Table 1 Angiotensin I converting enzyme (ACE) genotype and allele distributions in Italian, Spanish, and United Kingdom populations*

Nationality	Age at onset or collection (years), mean (SD)	Genotypes (n)			Alleles (n)	
		ACE*I/*I	ACE*I/*D	ACE*D/*D	ACE*I	ACE*D
Italian AD (n=141) Frequency (95% CI)	71 (8.5)	17 0.12 (0.08 to 0.19)	75 0.53 (0.45 to 0.61)	49 0.35 (0.27 to 0.43)	109 0.39 (0.33 to 0.45)	173 0.61 (0.56 to 0.67)
Spanish AD[4] (n=350) Frequency (95% CI)	72 (9.0)	70 0.20 (0.16 to 0.25)	161 0.46 (0.41 to 0.51)	119 0.34 (0.29 to 0.39)	301 0.43 (0.39 to 0.47)	399 0.57 (0.53 to 0.61)
UK AD[2] (n=239) Frequency (95% CI)	81.2 (7.8)	60 0.25 (0.20 to 0.31)	111 0.46 (0.40 to 0.53)	68 0.28 (0.23 to 0.35)	231 0.48 (0.44 to 0.53)	247 0.52 (0.47 to 0.56)
UK AD[1] (n=542) Frequency (95% CI)	70.3 (9.4); 82.3 (6.7); 76.6 (6.3)	127 0.23 (0.20 to 0.27)	323 0.60 (0.55 to 0.64)	92 0.17 (0.14 to 0.20)	577 0.53 (0.50 to 0.56)	507 0.47 (0.44 to 0.50)
Italian controls (n=268) Frequency (95% CI)	72 (7.1)	32 0.12 (0.08 to 0.16)	138 0.52 (0.46 to 0.57)	98 0.37 (0.42 to 0.31)	202 0.38 (0.34 to 0.42)	334 0.62 (0.58 to 0.66)
Spanish controls[4] (n=400) Frequency (95% CI)	21 to 65 (range)	60 0.15 (0.12 to 0.19)	176 0.44 (0.39 to 0.49)	164 0.41 (0.36 to 0.46)	296 0.37 (0.34 to 0.40)	504 0.63 (0.60 to 0.66)
UK controls[2] (n=342) Frequency (95% CI)	82.1 (3.8)	60 0.18 (0.14 to 0.22)	203 0.59 (0.54 to 0.64)	79 0.23 (0.19 to 0.28)	323 0.47 (0.44 to 0.51)	361 0.53 (0.49 to 0.57)
UK controls[1] (n=386) Frequency (95% CI)	73.5 (6.2); 80.8 (4.5); 77.1 (6.4)	89 0.23 (0.19 to 0.28)	180 0.47 (0.42 to 0.52)	117 0.30 (0.26 to 0.35)	358 0.46 (0.43 to 0.50)	414 0.54 (0.50 to 0.57)

n, number of individuals genotyped.
*Criteria for selection of published ACE frequencies were the sampling amplitude (>100 subjects) and the diagnosis of Alzheimer's disease made according to the same clinical criteria.
ACE, angiotensin I converting enzyme; AD, Alzheimer's disease; CI, confidence interval; I, ACE*I; D, ACE*D; II, ACE*I/*I; DD, ACE*D/*D; ID, ACE*I/*D.

evidence that the genotypic counts of Alzheimer's disease patients and controls were not under HWE (Alzheimer's disease patients under HWE, given the controls were under HWE: likelihood ratio, $\chi^2 = 2.18$, p = 0.34). No significant differences were found in ACE genotype frequencies between patients with Alzheimer's disease and controls in this southern Italian population.

We did not find any statistically significant differences in rates between ACE alleles and Alzheimer's disease among APOE allele strata, nor did we observe any lack of homogeneity among response differences (data not shown). Interestingly, Alzheimer patients with the ACE*I/*I genotype were on average 3.6 years younger at onset than those with the ACE*D/*D genotype (mean (SD) age at onset: ACE*D/*D, 72.1 (6.8) years; ACE*I/*D, 70.3 (8.1) years; ACE*I/*I, 68.5 (7.3) years). However, this difference did not reach statistical significance (z = 1.49; Bonferroni p > 0.05).

The ACE*I allele frequency in Alzheimer's disease patients and controls showed a statistically significant decreasing trend from northern to southern regions of Europe (z = 5.36 p <0.001; z = 4.33 p <0.001, respectively), while there was a concomitant increase in ACE*D allele frequency (table 1). This was reflected by genotype data whereby a decreasing geographical trend from north to south was found for ACE*I/*I genotype (cases, z = 3.92 p <0.01; controls, z = 4.15 p <0.001) and an inverse trend for ACE*D/*D genotype (cases, z = 3.29 p <0.001; controls, z = 3.46 p<0.001). Interestingly, we found a statistically significant decreasing trend from northern to southern regions for the ACE*I/*D genotype, but this

was observed only in Alzheimer's disease patients (z = 3.12 p <0.01).

Comment

The present study does not support previous findings that increased Alzheimer's disease risk is associated with the ACE*I genotype and allele frequencies.[1] The age at onset of Alzheimer's disease patients with the ACE*I/*I genotype appeared to be lower than in those with the ACE*D/*D genotype. Though this was not statistically significant, it suggests that the presence of an ACE*I allele might bring forward the onset of the disease without being linked to an increased overall risk of it occurring. Our findings support those of a previous report in which no evidence of an interaction between ACE alleles and age at onset, sex, and family history was found (data not shown).[1]

It is becoming apparent that the possible association between the ACE polymorphism and increased Alzheimer's disease risk is complex. The variation in results between different studies may simply reflect the inherent susceptibility of such association studies to type I and type II statistical errors. Another possible explanation may be the direct result of geographical genetic variation which we have hypothesised. Indeed, as with our previous findings with APOE,[6] we report here that the putative association between ACE gene variants and increased risk of Alzheimer's disease may be influenced by geographical genetic variations (table 1). The different and conflicting patterns of association of ACE polymorphism and Alzheimer's disease in populations worldwide may be explained by similar geographic trends or indeed another Alzheimer's

disease susceptibility locus located elsewhere in ACE or a nearby gene. Furthermore, the same ACE gene may have pleiotropic age and sex dependent effects on Alzheimer's disease. Though the strength of association of APOE e4 with Alzheimer's disease seems not to be influenced by the low prevalence of e4 in southern Europe,[6] the decrease of the ACE*I allele frequency could be related to the different patterns of association between this polymorphism and Alzheimer's disease in various European studies.[1–5]

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Table 1

Enzyme	Presumed genotype at risk	Total number		% at risk		OR	CI	p
		Cases	Controls	Cases	Controls			
NAT 2	2 mutant allels	150	373	62.0	57.6	1.2	0.8 to 1.8	0.2
GSTM1	2 mutant allels	150	373	49.3	51.1	0.9	0.6 to 1.4	0.4
	No GSTM1*A allele	150	373	68.0	64.1	1.2	0.8 to 1.8	0.3
	No GSTM1*B allele	150	373	76.7	84.7	0.6	0.3 to 0.9	0.01
GSTT1	1 or 2 active alleles	150	360	84.0	79.2	1.4	0.8 to 2.4	0.1
MEH	2 Tyr113 mutations	150	336	50.0	47.6	1.0	0.7 to 1.6	0.4
MEH	2 His139 mutations	150	330	60.0	63.3	0.8	0.6 to 1.3	0.2
CYP1A1	1 or 2 6235C mutations	150	349	11.3	14.9	0.7	0.4 to 1.3	0.2
CYP1A1	1 or 2 Val462 mutations	150	348	5.3	5.7	0.8	0.3 to 1.9	0.4
CYP2C19	1 or 2 active alleles	150	340	98.0	96.5	1.8	0.5 to 10.0	0.3
CYP2D6	1 or 2 active alleles	150	373	4.7	5.9	0.8	0.5 to 3.6	0.4
CYP2E1	1 or 2 1019T mutations	150	348	2.0	5.7	0.3	0.06 to 1.2	0.04
CYP2E1	1 or 2 9930G mutations	150	328	24.7	25.9	0.9	0.6 to 1.4	0.4
CYP2E1	1 or 2 7776T mutations	150	300	12.7	12.6	1.0	0.5 to 1.9	0.6

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Polymorphisms of toxifying and detoxifying hepatic enzymes in amyotrophic lateral sclerosis

A contribution of hepatic enzymes responsible for detoxification and toxification of xenobiotics and endogenous compounds has been suspected to contribute to the pathogenesis of amyotrophic lateral sclerosis (ALS). We studied 12 potentially relevant enzymes in 150 ALS patients and 373 controls on the genetic level and could not detect any significant difference between both groups. These results strongly support a view that—in contrast with earlier observations—hepatic foreign compound metabolism does not contribute to the pathogenesis of ALS.

Genetic studies of familial ALS have yielded at least six chromosomal loci and two disease genes ("alsin" and superoxide dismutase 1).[1–3] Mutations on the superoxide dismutase 1 initially suggested a role for free radicals in the disease process but recent results clearly argue for a gain of function mechanism. The mechanisms through which the mutant enzyme exerts toxicity and results in selective motor neuron death remain unclear. Although familial ALS accounts only for 2% of all cases, the findings on the DNA level demonstrate the significance of genetic factors.

In contrast, the cause of the sporadic form of ALS remains largely obscure. Basically, the aetiology of the disease is viewed as multifactorial with polygenic as well as ecological factors. Assuming an involvement of exogenous or endogenous toxic factors, an interindividually different capacity for toxification or detoxification of endogenous compounds, xenobiotics including drugs could cause an inter-individually different susceptibility to develop ALS. Thus, respective enzymes and their encoding genes with functionally different alleles might be candidates for susceptibility genes for the sporadic form of ALS. Some earlier studies of the metabolic phenotype seemed to show an altered xenobiotic metabolism in ALS patients. For example, Heafield *et al*[4] described 74% slow acetylators among 14 ALS patients compared with 60% in the normal population.

We investigated a number of different genes encoding for toxifying and detoxifying enzymes that have been suspected to be causally linked to ALS: arylamine-N acetyl-transferase (NAT2), the glutathione-S transferases (GSTs) M1 and T1, microsomal epoxide hydrolase (mEH) as well as the cytochrome P-450 enzymes (CYP) 1A1, 2E1, 2C19, and 2D6. For all these enzymes, well defined polymorphisms are known. All methods used have been described previously.[5] Briefly, DNA was extracted from blood samples, PCR amplified by gene specific primers, and analysed by restriction fragment length polymorphisms (RFLP).

We analysed blood of 150 patients with the diagnosis of sporadic ALS according to the revised El Escorial criteria and 373 control patients recruited in three German centres (Berlin, Homburg/Saar, and Hannover). Control patients had non-neurological diagnoses and were of white origin. The mean age of the patients was 55.6 years. The ratio of men to women was 1.1:1. In 26.7% of the patients the disease was of bulbar, in 73.3% of spinal onset.

Our RFLP analysis could not reveal any significant over-representation of a polymorphism (table 1) that has been associated with an altered metabolism for the encoded enzymes in ALS patients. In contrast with our hypothesis, we found a significant over-representation of the GST M1*B allele with 24% in the patients group versus 15.3% in the control group and a significant under-representation of the CYP2E1$_{1019T}$ mutation (table 1). However, in the absence of differences of activity between the isoenzymes GSTM1*A and GSTM1*B, the significance of the B-allelic over-representation among ALS patients remains uncertain. As the CYP2E1 has toxifying properties and the CYP2E1$_{1019T}$ mutation is associated with an increased enzyme activity an under-representation in the ALS population is likely to be of minor significance.

Our results are in accordance with other genotype studies analysing the enzymes GSTM1, CYP2D6, CYP1A1 and NAT2[6] as well as NAT2 and CYP2D6[7] where no significant differences between patients and control groups were found. Using a substantially larger population of ALS patients and extending these studies for other toxifying and detoxifying enzymes, we have found no significant differences between patients and control groups for the glutathione-S transferase T1, the microsomal epoxide hydrolase, and the cytochrome P-450 enzymes 2E1 and 2C19.

We conclude from our data, that an involvement of the analysed toxifying and detoxifying enzymes in the pathogenesis of ALS is most unlikely.

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Favourable outcome of a brain trauma patient despite bilateral loss of cortical somatosensory evoked potential during thiopental sedation

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