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 together with the comparison of whole genome linkage studies across these diseases 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, following on from which two independent genome-wide scans have yielded positive results. 

The work was supported by the Wellcome Trust (grant 057097).

Acknowledgements

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Competing interests: none declared
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Table 1 Observed frequency of Crohn’s disease associated alleles in multiple sclerosis

<table>
<thead>
<tr>
<th>Variant</th>
<th>Multiple sclerosis (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBDB8*</td>
<td>54 (4.8)</td>
<td>36 (6.2)</td>
</tr>
<tr>
<td>IBDB12</td>
<td>11 (0.9)</td>
<td>6 (0.9)</td>
</tr>
<tr>
<td>IBDB13</td>
<td>28 (2.3)</td>
<td>8 (1.2)</td>
</tr>
</tbody>
</table>

*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 IBDB where a lower annealing temperature of 50°C was used along with four additional PCR cycles.

Primary PCR primers

IBDB8: ACCTTCAGATCACAGCAGCC and GCTCCCCCATACCTGAAC
IBDB12: AAGTCTGTAATGTAAAGCCA and CCCAGCTCCTCCCTCTTC
IBDB13: TTTTTTTTTGGTGTCATTCCTTTCAAGGG

References


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.

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 E3V3M (9/15), with preserved pupillary reflexes and gross motor function. Computed tomography of the head showed a traumatic disjunction of the lamboid 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 arreative (GCS 3/15) and without brain stem reflexes. The patient’s SSEP patterns did not return to normal 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 moderate dysphasia and generalised weakness. At four months, she presented no residual motor deficit, an improved verbal expression and comprehension, and a moderate frontal motor deficit, an improved verbal expression and comprehension, and a moderate frontal
In humans supposedly do not disappear in response to barbiturate doses sufficient to render the EEG isoelectrical and the neurological examination similar to brain stem death.\(^1\) 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.\(^1\) In our case, the disappearance of the cortical evoked responses correlated with both the ICP increase and the induction of thiotepal coma. As their reappearance closely matched the elimination of thiotepal 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.

Funding: PAR and SL are post-doctoral researchers at the Fonds National de la Recherche Scientifique (FNRS).

References


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 MT-K 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.

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 in 100 000 in the adult population of Northern Ostrobothnia.\(^1\) 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 \(\geq 20\) years of age. Adult patients with diagnoses that are commonly associated with the 8344A>G mutation were identified as being at risk with respect to mitochondrial disorders, and we therefore screened the population for patients \(\geq 20\) 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

Table 1 Criteria used in the screening of the patient groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Selection criterion 1</th>
<th>Selection criterion 2</th>
<th>Number of patients identified</th>
<th>Number of samples received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia</td>
<td>Any ataxia, unknown aetiology</td>
<td>Idiopathic cerebellar ataxia, age (\geq 20) years at visit</td>
<td>79</td>
<td>169</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>Insulin treatment started at age 20–45 years</td>
<td>Family history of mitochondrial phenotype†</td>
<td>479</td>
<td>39</td>
</tr>
<tr>
<td>Epilepsy‡</td>
<td>Age (\geq 20) years at visit, response to family history questionnaire</td>
<td>Family history of mitochondrial phenotype†</td>
<td>945</td>
<td>223</td>
</tr>
<tr>
<td>Hearing loss§</td>
<td>Sensory-neural hearing impairment, hearing aid obtained at age (\geq 45) years, current age (\geq 20) years</td>
<td>Family history of mitochondrial phenotype†</td>
<td>242</td>
<td>108</td>
</tr>
<tr>
<td>Lipoma</td>
<td>Any lipoma</td>
<td>Axial or multiple lipomas, age (\geq 20) years at visit</td>
<td>621</td>
<td>150</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Any myopathy with clinical and EMG verification, age (\geq 20) years at visit</td>
<td>Myopathy of unknown aetiology or any muscle dystrophy‡</td>
<td>146</td>
<td>41</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>Any electrophysiologically defined idiopathic neuropathy, age (\geq 20) years at visit</td>
<td>Familial neuropathy or family history of mitochondrial phenotype†</td>
<td>138</td>
<td>31</td>
</tr>
<tr>
<td>Ophthalmoplegia</td>
<td>Double vision or ptosis, any age</td>
<td>Definite ophthalmoplegia or symmetric ptosis, age (\geq 20) years at examination</td>
<td>799</td>
<td>15</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>Decrease in visual acuity or optic disc abnormality, any cause, any age</td>
<td>Optic atrophy of unknown aetiology*, current age (\geq 20) years</td>
<td>1542</td>
<td>42</td>
</tr>
</tbody>
</table>

OUH, Oulu University Hospital. Computer search at OUH was first performed to identify patients with specific discharge diagnoses that had been listed 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 in 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 supplied was reviewed and patients were ascertained on the basis of the following clinical criteria: symmetric sensorineural hearing impairment with a frequency of 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.
negative selection and may explain the differences may therefore be faced with different
period, centre in United Kingdom over a 10 year
among adult patients in a single neurology
estimated frequency of 8344A>G in northern
thus calculated to be 0–1.5/100 000. The
3.67). The prevalence of 8344A>G in the adult
mutation (95% confidence intervals (CI) 0 to
MERRF (table 1), and samples obtained from
ascertained as described in detail previously.

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
denmark 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: 0.23/100 000 (95% CI 0.01 to 0.50)
among adult patients in a single neurology
centre in United Kingdom over a 10 year period,1 and 0 to 0.25/100 000 (95% CI) in a
population based study among children in western Sweden.3 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.3
The frequency of 3243A>G has been found to be four times that of 8344A>G in the United
Kingdom.1 Furthermore, gene analyses in a molecular diagnostic laboratory have revealed that these two mutations among 2000
patients with features of mitochondrial
disorders is 4, suggesting that the frequency
to the 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 ascertainment in a population based manner.

MIDNA 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 population1 and is not faced with
any strong selection pressure,5 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
mRNA but does not cause a D-loop
mutation.7 Evolutionarily, these two
mutations may therefore be faced with different
negative selection and may explain the
differences in population frequencies.8

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.9 Studies conducted in northern
Scandinavian populations1,9,10 supported the ACE1 allele to be a risk factor for various types of cognitive decline.11 One such study in a
French population found an association
between the ACE1D allele and dementia, while other studies in southern
European populations found either a slight
but significantly increased frequency of ACE1 in Alzheimer’s disease patients1 or did not detect any effect of ACE polymorphism.3

Our group recently reported the novel finding that apolipoprotein E (APOE) e4 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 be-
 tween ACE polymorphism and Alzheimer’s
disease was related to similar geographical
differences in ACE1 frequency. We investi-
gated whether there was evidence in southern
Italy of an association between the ACE poly-
orphism and increased risk of Alzheimer’s
disease. Secondly, we discussed our findings with the findings from published studies on other European populations.1,13

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, 50 women; mean age of onset, 75 (7.2)
years). A clinical diagnosis of probable
Alzheimer’s disease was made according to the
criteria of the National Institute for Neurologi-
 cal and Communicative Disorders and 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 were not described in detail elsewhere.1 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 committees of the
University of Oulu. After a complete explanation
of the study, written informed consent was
obtained from all the subjects or their relatives. ACE genotypes were not controlled for
tempered elsewhere.2 ACE genotypes were pro-
duced using established methods, followed by
a quality control amplification step necessary
in detecting underamplified ACE1 alleles.12

The statistical analysis was performed by
Pearson χ2 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 count-
ning. To express variances of the allele frequen-
cies, 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 02113). 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 frequen-
cies in Alzheimer’s disease patients and controls of three European countries (Italy,
Spain, and United Kingdom), from published studies.1,13 The data were analysed by SAS
FREQ procedure (version 8.2).

Table 1 shows ACE allele and genotypes frequen-
cies 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 χ2 = 2.09, p = 0.15; controls:
χ2 = 2.49, p = 0.11. Moreover, there was no

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ragged-red fiber mutation provides new
insights into human mitochondrial function and

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

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Age at onset or collection (years), mean (SD)</th>
<th>Genotypes (n)</th>
<th>Alleles (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACE*1/*1</td>
<td>ACE*1/*2</td>
</tr>
<tr>
<td>Italian AD (n=141)</td>
<td>71 (8.5)</td>
<td>17</td>
<td>75</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.12</td>
<td>0.53</td>
</tr>
<tr>
<td>ACE*1/*2</td>
<td></td>
<td>(0.08 to 0.19)</td>
<td>(0.45 to 0.61)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.20</td>
<td>0.46</td>
</tr>
<tr>
<td>ACE*2/*2</td>
<td></td>
<td>(0.16 to 0.25)</td>
<td>(0.45 to 0.61)</td>
</tr>
<tr>
<td>UK AD* (n=239)</td>
<td>81.2 (7.8)</td>
<td>60</td>
<td>111</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.25</td>
<td>0.46</td>
</tr>
<tr>
<td>ACE*1/*1</td>
<td></td>
<td>(0.20 to 0.31)</td>
<td>(0.40 to 0.53)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.23</td>
<td>0.60</td>
</tr>
<tr>
<td>ACE*1/*2</td>
<td></td>
<td>(0.20 to 0.27)</td>
<td>(0.55 to 0.64)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.12</td>
<td>0.52</td>
</tr>
<tr>
<td>ACE*2/*2</td>
<td></td>
<td>(0.08 to 0.16)</td>
<td>(0.46 to 0.57)</td>
</tr>
<tr>
<td>Spanish controls*</td>
<td>72 (7.1)</td>
<td>32</td>
<td>138</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.12</td>
<td>0.52</td>
</tr>
<tr>
<td>ACE*1/*1</td>
<td></td>
<td>(0.08 to 0.16)</td>
<td>(0.46 to 0.57)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.15</td>
<td>0.44</td>
</tr>
<tr>
<td>ACE*1/*2</td>
<td></td>
<td>(0.12 to 0.19)</td>
<td>(0.39 to 0.49)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.18</td>
<td>0.59</td>
</tr>
<tr>
<td>ACE*2/*2</td>
<td></td>
<td>(0.14 to 0.22)</td>
<td>(0.54 to 0.64)</td>
</tr>
<tr>
<td>UK controls† (n=342)</td>
<td>82.1 (3.8)</td>
<td>60</td>
<td>203</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.18</td>
<td>0.59</td>
</tr>
<tr>
<td>ACE*1/*1</td>
<td></td>
<td>(0.14 to 0.22)</td>
<td>(0.54 to 0.64)</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.23</td>
<td>0.47</td>
</tr>
<tr>
<td>ACE*1/*2</td>
<td></td>
<td>(0.19 to 0.28)</td>
<td>(0.42 to 0.52)</td>
</tr>
</tbody>
</table>

n, number of individuals genotyped.

Table 1. 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; ID.

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 not under HWE (Alzheimer’s disease patients and controls were not under HWE). Interestingly, we found a statistically significant trend for ACE*D/*D genotype (cases, p <0.01; controls, p <0.001 and an inverse trend for ACE*D/*D genotype (cases, p = 0.23 vs. controls, p = 0.46). Interestingly, we found a statistically significant decreasing trend from northern to southern regions for the ACE*D/*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 those with the ACE*D/*D genotype. Though this was not statistically significant, it presents the possibility 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). 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,2 we report here that the putative association between ACE gene variants and increased risk of Alzheimer’s disease may be influenced by geographical genetic variation (table 1). The different and conflicting patterns of association between ACE polymorphism and Alzheimer’s disease in populations worldwide may be explained by similar geographical 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 ε4 with Alzheimer’s disease seems not to be influenced by the low prevalence of ε4 in southern Europe,2 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 populations.2,3

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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. 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) at least six chromosomal loci and two disease genes (GSTM1, CYP2D6, CYP1A1 and NAT2) associated with an increased enzyme activity and 2D6. For all these enzymes, well defined polymorphisms are known. All methods used have been described previously. 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. 26.7% of the patients the disease was of bulb, 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 enzyme 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*1*1 mutation (table 1). However, in the absence of differences of activity between the isoforms 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*1*1 mutation is associated with an increased enzyme activity, an under-representation in the ALS population is likely to be of minor significance.

Table 1

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<tr>
<th>Enzyme</th>
<th>Presumed genotype at risk</th>
<th>Total number</th>
<th>% at risk</th>
<th>OR</th>
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<td>Controls</td>
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References