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 autoimmune diathesis in general and others determining precisely which autoimmune phenotype may result. On this basis it is reasonable to hypothesize 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 pericentric region of chromosome 16 has been found, followed on from two independent genome scans and association mapping and the other following a candidate gene approach, identified the relevant gene as NOD2. Three variants of this gene (IBD8, IBD12, IBD13) were shown to influence susceptibility to Crohn’s disease: IBD8 is a missense mutation in exon 3 (2023C>T, R675W); IBD12 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.


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 sedation.

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 E3V5M (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 cephalospinal 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 astatic (GCS 3/15) and without brainstem reflexes. Spontaneous, vigil- 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 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.

References


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>
<th>Published control frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD8*</td>
<td>54 (4.8)</td>
<td>34 (6.2)</td>
<td>4</td>
</tr>
<tr>
<td>IBD12</td>
<td>11 (0.9)</td>
<td>6 (0.9)</td>
<td>1</td>
</tr>
<tr>
<td>IBD13</td>
<td>28 (2.3)</td>
<td>8 (1.2)</td>
<td>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 for 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: ACCCTCGACTACGAGCGG and GCTCCCTACATTCTGAAAC
IBD12: AGATCCTGAATGAATGGAAGCA and CGCTCCCTCCTTCC
IBD13: CTACATTGATCTCCTTTCTC and GAAGTCGACGAGCAAGGG

Extension primers

IBD1: TTCTTGGTCTAGCTAGAGAGC
IBD2: TGCTTCTTGAAGC
IBD3: TTITITGTTGTCAGCTCTCCAAAGG

Acknowledgements

We thank members of the Association of British Neurologists and the Multiple Sclerosis Society of Great Britain and Northern Ireland for notifying us of the patient in this genetic analysis of multiple sclerosis. The work was supported by the Wellcome Trust (grant 057097).

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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.\(^2\) 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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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 encephalomyopa thy caused by a 8344A>G mutation in the MT-TK 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.\(^3\)

The population frequencies of pathogenic mutations in mitochondrial DNA (mtDNA) are not well known, but the Finnish health care 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 1:16 000 in the adult population of Northern Ostrobothnia.\(^4\) 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 8343A>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 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

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Selection criterion 1</th>
<th>Number of patients identified</th>
<th>Selection criterion 2</th>
<th>Number of patients identified</th>
<th>Number of samples received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axatia</td>
<td>Any axatia, unknown aetiology</td>
<td>79</td>
<td>Idiopathic cerebellar axatia, age ≥20 years at visit</td>
<td>39</td>
<td>26 (67)</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>Insulin treatment started at age 20–45 years</td>
<td>479</td>
<td>Family history of mitochondrial phenotype†</td>
<td>169</td>
<td>143 (85)</td>
</tr>
<tr>
<td>Epilepsy‡</td>
<td>Age ≥20 years at visit, response to family history questionnaire</td>
<td>945</td>
<td>Family history of mitochondrial phenotype†</td>
<td>223</td>
<td>165 (74)</td>
</tr>
<tr>
<td>Hearing loss$</td>
<td>Sensorineural hearing impairment, hearing aid obtained at age ≤45 years, current age ≥20 years</td>
<td>242</td>
<td>Family history of mitochondrial phenotype†</td>
<td>108</td>
<td>82 (76)</td>
</tr>
<tr>
<td>Lipoma</td>
<td>Any lipoma</td>
<td>621</td>
<td>Axial or multiple lipomas, age ≥20 years at visit</td>
<td>150</td>
<td>107 (71)</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Any myopathy with clinical and EMG verification, age ≥20 years at visit</td>
<td>146</td>
<td>Myopathy of unknown aetiology or any muscle dystrophy§</td>
<td>41</td>
<td>32 (78)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>Any electrophysiologically defined idiopathic neuropathy, age ≥20 years at visit</td>
<td>138</td>
<td>Familial neuropathy or family history of mitochondrial phenotype‡</td>
<td>31</td>
<td>21 (68)</td>
</tr>
<tr>
<td>Ophthalmoplegia</td>
<td>Double vision or ptosis, any age</td>
<td>799</td>
<td>Definite ophthalmoplegia or symmetric ptosis, age ≥20 years at examination</td>
<td>15</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>Decrease in visual acuity or optic disc abnormality, any cause, any age</td>
<td>1542</td>
<td>Optic atrophy of unknown aetiology*, current age ≥20 years</td>
<td>42</td>
<td>30 (71)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4991</td>
<td>Total</td>
<td>818</td>
<td>621 (76)</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 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 local hospitals in the area and the diabetes register of the local authority health service 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 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. ††The epidemiological register of the myocardial infarction in Southern Ostrobothnia was surveyed. Patient with the following clinical criteria: symmetric sensorineural hearing impairment with 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. \(\cdot\)Duchenne muscular dystrophy and other myopathies with definite molecular genetic diagnosis were excluded. **Demyelinating diseases and ischaemic diseases were excluded.

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...as described in detail previously. 3 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 High Pure PCR Template Preparation Kit (Qiagen). A fragment encompassing the mitochondrial gene was amplified by PCR in the presence of 5'-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 MELAS (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 0.50%) among adult patients in a single neurology centre in United Kingdom over a 10 year period. The prevalence of 8344A>G in the adult population of Northern Ostrobothnia was thus calculated to be 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, and 0 to 0.25/100 000 (95% CI) in a population based study among children in western Sweden. 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. 3 Furthermore, gene analyses in a molecular diagnostic laboratory have revealed that these two mutations are among 2000 patients with features of mitochondrial disorders is 4, suggesting that the frequency ratio between the two is fairly constant. The 3243A>G MELAS mutation appears to be considerably more common than 8344A>G also among Finnish patients that was ascertained in a population based manner.

MITDNA 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 1778G>A, 3243A>G and 3460G>A, while 8344A>G is infrequent. The 3243A>G mutation has arisen several times in a population and is not faced with any strong selection pressure, 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 than does the MELAS mutation. 5 Evolutionarily, these two mutations may therefore be faced with different negative selection and may explain the differences in population frequencies.

**Acknowledgements**

The authors thank Ms Anja Heikkinen for her expert technical assistance. This study was supported by grants from the Medical Research Council of the Academy of Finland and the Sigrid Juselius Foundation.

**References**


**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.** Studies conducted in northern European population showed that the ACE*D allele is a risk factor for various types of cognitive decline. 4 One such study in a French population found an association between the ACE*D allele and dementia, while other studies in southern European populations found either a slight but significantly increased frequency of ACE*E in Alzheimer's disease patients 5 or did not detect any effect of ACE polymorphism. 6 Our group recently reported the novel finding that apolipoprotein E (APOE) 64 allele shows a geographical trend, decreasing in frequency from northern to southern Europe. 7 We hypothesised that the variability in the strength of evidence for an association between ACE polymorphism and Alzheimer's disease is related to similar geographical variations in ACE1 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 discussed our results with the findings from published studies on other European populations. 8–11 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 (SD) age at recruitment, 70 (8.7) 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 occurred in detail elsewhere. 8 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. ACE genotypes were available in 44% of cases and 59% of controls, as mentioned elsewhere. 8 ACE genotypes were produced using established methods, followed by a quality control amplification step necessary in determining underamplified ACE*E alleles. 6 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 counting. To express variances of the allele frequencies, we used 95% CIs, calculated by Wilson's formula. 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 (ProcLogXact 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 from three European countries (Italy, Spain, and United Kingdom), from published studies. 8–11 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 χ2 = 2.09, p = 0.15; controls: χ2 = 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

<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*I/*I</td>
<td>ACE*I/*D</td>
</tr>
<tr>
<td>Italian AD (n=141)</td>
<td>71 (8.5)</td>
<td>17</td>
<td>75</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.12 (0.08 to 0.19) 0.53 (0.45 to 0.61) 0.35 (0.27 to 0.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spanish AD (n=350)</td>
<td>72 (9.0)</td>
<td>70</td>
<td>161</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.20 (0.19 to 0.25) 0.46 (0.41 to 0.51) 0.34 (0.29 to 0.39)</td>
<td></td>
<td></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 (95% CI)</td>
<td>0.25 (0.20 to 0.31) 0.46 (0.40 to 0.53) 0.28 (0.23 to 0.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italian controls (n=268)</td>
<td>72 (7.1)</td>
<td>32</td>
<td>138</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.12 (0.08 to 0.16) 0.52 (0.46 to 0.57) 0.37 (0.42 to 0.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spanish controls (n=400)</td>
<td>21 to 65 (range)</td>
<td>60</td>
<td>176</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.15 (0.12 to 0.19) 0.44 (0.39 to 0.49) 0.41 (0.36 to 0.46)</td>
<td></td>
<td></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 (95% CI)</td>
<td>0.18 (0.14 to 0.22) 0.59 (0.54 to 0.64) 0.23 (0.19 to 0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK controls (n=386)</td>
<td>73.5 (6.2); 80.8 (4.5); 77.1 (6.4)</td>
<td>89</td>
<td>180</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.23 (0.19 to 0.28) 0.47 (0.42 to 0.52) 0.37 (0.26 to 0.35)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n, number of individuals genotyped.

References


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, χ² = 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, though differences in rates between ACE alleles and age at onset, sex, and family history was found (data not shown). Interestingly, Alzheimer patients with the ACE*I/*I genotype were on average 29 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/*I, 70.3 (8.1) years). How-ever, 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 = 4.35 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 < 0.001; controls, z = 4.15 p < 0.001) and an increasing 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 gene and allele frequencies. 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 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). 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, 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 between 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 influ-enced by the low prevalence of e4 in southern Europe, the decrease of the ACE*I allele frequency could be related to the different patterns of association between this polymorph-ism and Alzheimer’s disease in various European populations.1–5

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Competing interests: none declared

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Table 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Presumed genotype at risk</th>
<th>Total number</th>
<th>% at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>OR</td>
</tr>
<tr>
<td>NAT2</td>
<td>2 mutant alleles</td>
<td>150</td>
<td>373</td>
</tr>
<tr>
<td>GSMT1</td>
<td>2 mutant alleles</td>
<td>150</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>No GSTM1 *A allele</td>
<td>150</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td>No GSTM1 *B allele</td>
<td>150</td>
<td>373</td>
</tr>
<tr>
<td>GST1</td>
<td>1 or 2 active alleles</td>
<td>150</td>
<td>360</td>
</tr>
<tr>
<td>MEH</td>
<td>2 Ty1113 mutations</td>
<td>150</td>
<td>336</td>
</tr>
<tr>
<td>MEH</td>
<td>2 His139 mutations</td>
<td>150</td>
<td>330</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>1 or 2 6235C mutations</td>
<td>150</td>
<td>349</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>1 or 2 Val402 mutations</td>
<td>150</td>
<td>348</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1 or 2 active alleles</td>
<td>150</td>
<td>340</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>1 or 2 1019T mutations</td>
<td>150</td>
<td>348</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>1 or 2 9930G mutations</td>
<td>150</td>
<td>328</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>1 or 2 7776T mutations</td>
<td>150</td>
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Polymorphisms of toxifying and detoxifying hepatic enzymes in amyotrophic lateral sclerosis

A contribution of hepatic enzymes responsible for toxification and xenobiotic 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’). 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 etiology of the disease is viewed as multifactorial with polygenic as well as ecological factors. Assuming an involvement of exogenous or endogenous toxic factors, an interindividual 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, Heafied et al 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-acetyltransferase (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. 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 *1 *1 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 *1 *1 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 genotypic studies analysing the enzymes GSTM1, CYP2D6, CYP1A1 and NAT2 as well as NAT2 and CYP2D6 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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References


