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 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 pericentric region of chromosome 16 has been found, followed on from which two independent genome-wide association mapping studies have identified the following regions: the first (IBD12) in the pericentromeric region of chromosome 16 (2641G>C, G1881R); and IBD13 is a frameshift variant in exon 10 (2936insC, Δ2937C). The role of these three variants with susceptibility to Crohn’s disease was confirmed in a second by genotyping patients participating in this genetic analysis of multiple sclerosis. The work was supported by the Wellcome Trust (grant 05797).

S. Sawyer, M. Maranian, A. Hensiek, R. Roxburgh, J. Gray, A. Compston
University of Cambridge Neurology unit, Addenbrooke’s Hospital, Hills Road, Cambridge, CB2 0QX, UK
Competing interests: none declared
Correspondence to: Professor Alastair Compston; alastair.compston@medschl.cam.ac.uk

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 patients participating in this genetic analysis of multiple sclerosis. The work was supported by the Wellcome Trust (grant 05797).

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 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 E 3, V, M (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 iso-electrical and on day 11, somatosensory evoked potentials (SEP) of the median nerve showed no cortical response (N20) despite normal brachial plexus (Erb) and luminal (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 3/5) and without brain stem reflexes, pupillary, or autonomic response, until day 20. The transcranial Doppler however showed normal flow patterns and the brain CT scan did not reveal any post-herald ischemic 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 mild 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 suffered some episodic dysphasia. 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 coma-like patients even under barbiturative sedation. Indeed, although their morphological change can become altered, short latency SSEPs

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 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: ACCTCACGATACGACCAAGC GC and GCTCCCCTCTACCTGTAAC
IBD12: AGCTGCTAATGTAAGGCCA and CCCAGCCTCCCTCCTTIC
IBD13: CTCACCATGATCTTCTITTG and GAATGCGAAGCTACAGAAGG

Extension primers

IBD8: TTTTTTCTACGAGAAGGAGGCTGCTCTC
IBD12: TGGCTCTTCTACGATCG
IBD13: TTTTTTGTGGTCTACCTTTCAAGG

www.jnnp.com

J Neurol Neurosurg Psychiatry: first published as 10.1136/jnnp.74.8.1157 on 21 July 2003. Downloaded from http://jnnp.bmj.com/ on June 18, 2022 by guest. Protected by copyright.
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.

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.

In our case, the disappearance of the cortical evoked responses correlated with both the ICP increase and the induction of thiopeptol coma. As their reappearance closely matched the elevation of thiopeptol 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).

P A Robe, A Dubuisson
Service of Neurosurgery, University Hospital of Liège, Liège, Belgium

S Bartsch, P Damas
Service of Critical Care Medicine, University Hospital of Liège

S Laureys
Service of Neurology, University Hospital of Liège

Correspondence to: Dr P A Robe, Department of Neurosurgery, CHU de Liège, Domaine universitaire du Sart Tilman, 835, 4000 Liège, Belgium; pierre.robe@ulg.ac.be

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 MTK 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 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 acids, and stroke-like episodes), to be 16.6 in 10,000 in the adult population of Northern Ostrobothnia. 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

---

**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>67 (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>85 (82)</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>213</td>
<td>74 (82)</td>
</tr>
<tr>
<td>Hearing loss§</td>
<td>Sensorineural hearing impairment, hearing aid obtained at age &lt;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>71 (100)</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 a muscle dystrophy¶</td>
<td>41</td>
<td>78 (32)</td>
</tr>
<tr>
<td>Neoplasticity</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>68 (21)</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>100 (15)</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>Decrease in visual acuity or optic disc abnormality, any age, any age</td>
<td>1542</td>
<td>Optic atrophy of unknown aetiology***, current age ≥20 years</td>
<td>42</td>
<td>71 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4991</td>
<td>Total</td>
<td>818</td>
<td>76 (82)</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 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 with 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 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.¹ 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 10³-10⁴ ng/μl of primer. The 3844A>G mutation was detected by restriction fragment analysis using BglII. 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 syndrome (table 1), and samples obtained from 621 of these were examined for the 3844A>G mutation. None of the patients harboured the mutation (95% confidence intervals (CI) 0 to 3.67). The prevalence of 3844A>G in the adult population of Northern Ostrobothnia was thus calculated to be 0–1.5/100 000. The population of Northern Ostrobothnia was 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.² The frequency of 3243A>G has been found in northern Sweden (table 1), and samples obtained from 621 of these were examined for the 3844A>G mutation. None of the patients harboured the mutation (95% confidence intervals (CI) 0 to 3.05) among adult patients in a single neurology department in Finland is much lower than that of 3243A>G, thus calculated to be 0–1.5/100 000. The most common mtDNA point mutation (95% confidence intervals (CI) 0 to 1.50) 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 west London.⁴ The 3844A>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.¹

The frequency of 3243A>G has been found to be four times that of 8344A>G in the United Kingdom.² 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 ratio between the two is fairly constant. The 3243A>G-MELAS mutation appears to be clearly more common than 3844A>G also among Finnish patients that was ascertained in a population based manner.

MIDNA mutations are a comparatively common cause of neurodegenerative disorders in both adults and children, but they vary in prevalence. The most common mtDNA point mutations seem to suffer from a high frequency of underamplification, as suggested by the QIAGEN kit (Qiagen) and the 3460G>A, while 8344A>G is infrequent. The 3243A>G mutation has arisen several times in a population⁵ and is not faced with any strong selection pressure;¹ 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 machinery, whereas the 3844A>G does the polymorphism.¹ Evolutionarily, these two mutations may therefore be faced with different negative selection and may explain the differences in population frequencies.¹

References

3. Chinnery PF, Johnson MA, Wardell TM, et al. 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 variations in 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 logictic 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 the 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.¹ The data were analysed by SAS FREQ procedure (version 8.2).

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.⁶ Studies conducted in northern Scandinavian populations showed that the ACE*E allele is a risk factor for various types of cognitive decline.¹ ¹ One such study in a French population found an association between the ACE*E allele and dementia,⁶ while other studies in southern European populations found either a slight but significantly increased frequency of ACE*E in Alzheimer’s disease patients⁶ or did not detect any effect of ACE polymorphism.¹

Our group recently reported the novel finding that apoliprotein E (APOE) e4 allele shows a geographical trend, decreasing in frequency from northern to southern Italy.¹ ¹ We hypothesised that the variability in the strength of evidence for an association between ACE polymorphism and Alzheimer’s disease is related to similar geographic variations in ACE*E frequency. We investigated whether there was evidence in southern Italy of an association between the ACE polymorphism and increased risk of Alzheimer’s disease.¹ ¹ Second, we discussed whether our results were in line with the findings from published studies on other European populations.¹ ¹

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 onset, 70 (8.2) 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.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 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 determined in two different laboratories elsewhere:¹ ACE genotypes were produced using established methods, followed by a quality control amplification step necessary in detecting underamplified ACE*E alleles.¹ ¹

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 variations in 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 logictic 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 the 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.¹ 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
Alzheimer's disease patients and controls showed a statistically significant difference in the frequency of the ACE*I allele (data not shown). The ACE*I allele frequency in Alzheimer's disease patients was higher than in controls (cases, z = 2.02, p < 0.05; controls, z = 1.53, p = 0.12). 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 (cases, z = -2.37, p < 0.01; controls, z = -1.93, p = 0.05). This was reflected by genotype data whereby a decreasing geographical trend from north to south was found for ACE*I/*I genotype (cases, z = -2.35, p < 0.01; controls, z = -1.91, p = 0.05) and an inverse trend for ACE*D/*D genotype (cases, z = -1.62, p = 0.10; controls, z = -1.37, p = 0.17). However, this difference did not reach statistical significance (z = 1.49, Bonferroni p > 0.05).

Table 1

<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</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)</td>
<td>0.53 (0.45 to 0.61)</td>
<td>0.35 (0.27 to 0.43)</td>
</tr>
<tr>
<td>Spanish AD</td>
<td>72 (9.0)</td>
<td>70</td>
<td>161</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.20 (0.16 to 0.25)</td>
<td>0.46 (0.39 to 0.51)</td>
<td>0.34 (0.39 to 0.39)</td>
</tr>
<tr>
<td>UK AD</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 0.30)</td>
<td>0.46 (0.40 to 0.53)</td>
<td>0.28 (0.23 to 0.35)</td>
</tr>
<tr>
<td>UK AD</td>
<td>70.3 (9.4)</td>
<td>127</td>
<td>323</td>
</tr>
<tr>
<td>Frequency (95% CI)</td>
<td>0.23 (0.20 to 0.27)</td>
<td>0.60 (0.55 to 0.64)</td>
<td>0.17 (0.14 to 0.20)</td>
</tr>
<tr>
<td>Italian controls</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)</td>
<td>0.52 (0.46 to 0.57)</td>
<td>0.37 (0.42 to 0.31)</td>
</tr>
<tr>
<td>Spanish controls</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)</td>
<td>0.44 (0.39 to 0.49)</td>
<td>0.41 (0.36 to 0.46)</td>
</tr>
<tr>
<td>UK controls</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)</td>
<td>0.59 (0.54 to 0.64)</td>
<td>0.47 (0.19 to 0.28)</td>
</tr>
<tr>
<td>UK controls</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)</td>
<td>0.47 (0.42 to 0.52)</td>
<td>0.40 (0.26 to 0.35)</td>
</tr>
</tbody>
</table>

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; D, ACE*D.

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 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).3

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,4 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 influenced by the low prevalence of e4 in southern Europe,5 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.6

F Panza, V Solfrizzi, A D’Introno, A M Colaciccio, C Capurso, A Capurso
Department of Geriatrics, Centre for Aging Brain, Memory Unit, University of Bari, Policlinico, Piazza Giubilo Cesare 11, 70124 Bari, Italy

P G Kehoe
Department of Care of the Elderly, University of Bristol, Frenchay Hospital, Bristol, UK

Competing interests: none declared
Correspondence to: Dr Francesco Panza; geriat df@geriatria.uniba.it

References

4 Alvarez R, Alvarez V, Lahoz CH, et al. Angiotensin converting enzyme and

www.jnnp.com

J Neurol Neurosurg Psychiatry 2003;74:1157–1161

Published online as 10.1136/jnnp.74.8.1161 on 21 July 2003. Downloaded from http://jnnp.bmj.com/ on June 18, 2022 by guest. Protected by copyright.


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) 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 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, Heafield 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. 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*mut 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*mut 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 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.

R Bachus
Department of Neurology, Humboldt University of Berlin, Germany

K Neubert, I Roots
Department of Clinical Pharmacology, Humboldt University of Berlin

J Prudlo
Department of Neurology, University of Homburg, University of Goettingen, Germany

J Brockmøller
Department of Clinical Pharmacology, University of Goettingen

A C Ludolph
Department of Neurology, University of Ulm, Germany

Correspondence to: Professor A C Ludolph, Department of Neurology, University of Ulm, 89075 Ulm, Stainhoferstraße 9, Germany; albert.ludolph@hu.de

References


Table 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Presumed genotype at risk</th>
<th>Total number</th>
<th>% at risk</th>
<th>OR</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2</td>
<td>2 mutant alleles</td>
<td>150</td>
<td>62.0</td>
<td>1.2</td>
<td>0.8 to 1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>GSTM1</td>
<td>2 mutant alleles</td>
<td>150</td>
<td>62.0</td>
<td>1.2</td>
<td>0.8 to 1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>GSTM1*A allele</td>
<td>150</td>
<td>68.0</td>
<td>1.2</td>
<td>0.8 to 1.8</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>GSTM1*B allele</td>
<td>150</td>
<td>64.0</td>
<td>1.2</td>
<td>0.8 to 1.8</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td>1 or 2 active alleles</td>
<td>150</td>
<td>76.7</td>
<td>0.6</td>
<td>0.3 to 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>MEH</td>
<td>2 Typ I 13 mutations</td>
<td>150</td>
<td>84.0</td>
<td>1.4</td>
<td>0.8 to 2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>MEH</td>
<td>2 His 139 mutations</td>
<td>150</td>
<td>79.2</td>
<td>1.4</td>
<td>0.8 to 2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>1 or 2 6335C mutations</td>
<td>150</td>
<td>11.3</td>
<td>0.7</td>
<td>0.4 to 1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>1 or 2 Val405 mutations</td>
<td>150</td>
<td>14.9</td>
<td>0.7</td>
<td>0.4 to 1.3</td>
<td>0.2</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1 or 2 active alleles</td>
<td>150</td>
<td>1.0</td>
<td>0.6</td>
<td>0.3 to 1.9</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1 or 2 1019T mutations</td>
<td>150</td>
<td>1.0</td>
<td>0.6</td>
<td>0.3 to 1.9</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>1 or 2 active alleles</td>
<td>150</td>
<td>4.0</td>
<td>0.3</td>
<td>0.06 to 1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>1 or 2 9390G mutations</td>
<td>150</td>
<td>2.0</td>
<td>0.3</td>
<td>0.06 to 1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>1 or 2 7776T mutations</td>
<td>150</td>
<td>2.0</td>
<td>0.3</td>
<td>0.06 to 1.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>