LETTERS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Patients and methods

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

Table 1 Criteria used in the screening of the patient groups

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

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