**LETTERS**

**Anticipation in familial amyotrophic lateral sclerosis with SOD1-G93S mutation**

A myotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the degeneration of motor neurons in the spinal cord, brain stem, and motor cortex, resulting in paralysis of limb, bulbar, and respiratory muscles. About 10% of ALS show a familial trait, and up to 20% of familial ALS is caused by missense mutations of Cu/Zn superoxide dismutase (SOD1). More than 70 mutations have been reported, including a mutation hotspot at codon 93. Mice expressing human mutant SOD1 develop age dependent ALS-like neurological symptoms and pathological features of motor neuron degeneration and cytoplasmic inclusions consisting of mutant SOD1. Patients with SOD1 mutations represent divergent phenotypes, including age of onset, duration of disease, and clinical symptoms, mostly depending on the nature of SOD1 mutation. Acceleration of the age of onset in successive generations called anticipation has been reported in the misense mutations at codon 84 (L84F and L84V) in the families with SOD1 mutations in Japan, United States, and Italy. We experienced anticipation of age at onset in Japanese families with SOD1-G93S mutation. In the families with the G93S mutation, age of onset became younger in the patients of successive generations, exhibiting anticipation (fig 1). We estimated the degree of anticipation of onset age in nine parent-offspring pairs from four Japanese families with G93S mutation of SOD1 (fig 1). The mean age of onset was 64.4 (SD 6.30) years in the parents, against 44.8 (SD 12.1) years in the offspring in the patients. The mean difference in age of onset in the parent-offspring pairs was 19.6 (SD 10.4) years in the G93S families, showing a statistical significance (p=0.0085 by paired t test and p=0.0077 by Wilcoxon test; fig 1). Thus, the age of onset accelerated in successive generations in the patients with G93S mutation. In addition, the duration of diseases with G93S mutation was slightly longer in the children than in the parents, although the difference was not significant (fig 1). Six amino acid substitutions (Ser [S], Val [V], Asp [D], Ala [A], Cys [C], and Arg [R]) have been known at glycine 93 of SOD1. Position 93 is located at the apex of a β hairpin joining two β strands of the SOD1 monomer, and it is critical for the stability of the backbone conformation of SOD1. These substitutions are all of the possible single base changes in codon 93, as the changes in the third position of the codon conserve its coding for glycine. However, the age of onset of patients with other mutations at codon 93 such as G93A mutation remained uniform.3 The patients with G93S mutation present a relatively late onset with a long clinical course, compared with those with G93A mutation;G93S v G93A; 51.9 (SD 14.9) and 43.1 (SD 16.6) years in onset age; and 7.1 (SD 3.1) and 2.4 (SD 1.4) years in disease duration.4 The present results imply that different amino acid substitution at codon 93 resulted in different phenotypes, but anticipation could be a unique feature in familial ALS with G93S mutation. It is still possible that anticipation is due to observer bias in that one does not know whether other offspring are going to get the disease later but are not affected at this stage. Although the mutation testing in all unaffected members is necessary to completely solve this issue, this is somewhat difficult because of ethical problems. At least the eldest sisters in families 1 and 3 (fig 1), who are alive without any symptoms over the age of onset of their parents, are shown to have no mutation of SOD1, further supporting the present view and alleviating the observer bias. Although anticipation has been reported in several neurodegenerative diseases, including many of the polyglutamine diseases, familial amyloidotic polyneuropathy (FAP) with V30M mutation of transthyretin and Creutzfeldt-Jakob disease (CJD) with E200K mutation of prion, the molecular basis for anticipation is understood only in the polyglutamine diseases with instability of CAG repeat expansion.5 It is unknown whether the mechanism for anticipation is the result of an additional genetic effect or of a related environmental factor. Anticipation was documented in the particular codons of the target proteins in FAP and CJD, as well as in familial ALS, suggesting the presence of genetic background, which interacts with particular codon mutations. The G93S mutation was reported almost exclusively in Japan, whereas other glycine 93 mutations were demonstrated elsewhere. It would be of interest to compare our results with the G93S mutation in other countries. Factors that generate anticipation of the G93S mutation might be related to the ethnic genetic determinants in addition to the difference of amino acid substitution at position 93, exacerbating the conformational abnormality of mutant SOD1 between successive generations.

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**Table 1** Age differences at disease onset and duration between the parent and offspring generation (n = 9, mean (SD, SEM))

<table>
<thead>
<tr>
<th></th>
<th>Parent</th>
<th>Offspring</th>
<th>Paired difference</th>
<th>p Value (paired difference in age of onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>64.4 (6.30, 2.10)</td>
<td>44.8 (12.1, 4.06)</td>
<td>19.6 (10.4, 3.48)</td>
<td>0.0005* / 0.0077†</td>
</tr>
</tbody>
</table>

**Figure 1** Pedigrees and anticipation of familial ALS with SOD1-G93S mutation. The nine parent-offspring pairs in four families (A, B, C, and D) were subjected to statistical analysis. The left and right sided numbers indicate age at onset and years of disease duration. The probands are indicated by arrows. Age differences at disease onset and duration between the parent and offspring generation (E) are calculated for the four families including the family (D) data, reprinted from J Neurol Sci with permission from Elsevier Science, and expressed as mean (SD, SEM). *Paired t test; †Wilcoxon test.
Apolipoprotein E4 in the temporal variant of frontotemporal dementia

Although the apolipoprotein E4 (apoE4) allele has consistently been associated with Alzheimer’s disease and other types of dementia, its association with frontotemporal dementia (FTD) is controversial. After our report in 1997 of increased apoE4 allele frequencies in sporadic FTD and its effect on the age at onset, other studies of cases of FTD with pathological confirmation or tau mutations did not confirm this effect.1 However, recently it has been shown that semantic dementia, the temporal variant of FTD, is associated with higher frequencies of the apoE4 allele.2 Therefore, we have genotyped apoE in our expanded FTD patient population and have assessed whether patients with predominance of temporal atrophy have higher frequencies of the apoE4 allele.

Patients were ascertained through a clinicopathological survey of patients with FTD in The Netherlands.3 We identified 111 patients with the diagnosis of probable FTD, established according to the Lund and Manchester criteria. Thirteen of the patients had an autosomal dominant form (defined as at least three affected family members in two generations) of FTD, with tau mutations identified in 10 (P301L, G272V, R406W, and A4280), and were excluded from further analyses. Predominant temporal atrophy, semiquantitatively assessed on CT and/or MRI, was found in 31 (32%) patients, whereas frontal atrophy with or without temporal atrophy was present in 67 (68%) patients. Nine of the 31 patients (29%) with temporal atrophy fulfilled the criteria for semantic dementia, and four patients (13%) showed severe problems in language comprehension, although the diagnosis of semantic dementia could not be definitively established due to incomplete or inconclusive neuropsychological testing. The clinical diagnosis of FTD was pathologically confirmed in all 17 patients who came to postmortem (five of whom had predominant temporal atrophy). Non-demented control subjects (n=561) were taken from the Rotterdam study.1 All patients and controls were genotyped for the apoE allele as described by Slooter et al.4 Both genotype frequencies and apoE4 allele frequencies were calculated for each group and compared with non-demented controls using a \( \chi^2 \) test.

Six per cent of the 98 patients with sporadic FTD had the apoE4/4 genotype, compared with 2.3% of non-demented controls (p=0.04). This genotype was present in 9.7% of patients with the temporal variant of FTD (p=0.01) compared with non-demented controls), compared with only 4.5% in patients with frontotemporal atrophy (p=0.5). Genotype frequencies of heterozygote E4 (E4/4) and homozygote E4 (E4/E4) carriers are summarised in table 1. The frequency of the apoE4 allele in all patients with sporadic FTD was 21.9%, compared with 15.3% in the non-demented controls (p=0.02). In patients with temporal atrophy the apoE4 allele frequency was as high as 29.0% (p=0.004), whereas in the patients with frontotemporal atrophy only 18.7% (p=0.3) of alleles was apoE4. No association between ApoE4 and the age at onset, nor the duration of symptoms, was found in the overall group, nor in the subgroups.

Our results show that the apoE4 allele frequency is increased in patients with the temporal variant of FTD compared with non-demented controls. Although a biological hypothesis justifying such an association is still lacking, the effect of the apoE4 allele on the predominance of temporal atrophy compared with frontal atrophy has also been observed in patients with Alzheimer’s disease.5 To verify the association between the apoE4 allele and the temporal variant of FTD, a large study with pathological confirmation of the clinical diagnosis of FTD is required to exclude admixture of patients with Alzheimer’s disease.6 However, in all 17 patients who were necropsied in our series, including five patients with temporal lobe atrophy, the clinical diagnosis was neuropathologically confirmed. This shows that the clinical criteria according to the Lund and Manchester groups, when combined with neuromaging and psychometric evaluation, are highly accurate. We conclude that the association we previously found between the apoE4 allele and sporadic FTD may be due to a selective increase of this allele in patients with the temporal variant of FTD.

### References


**Transferrin C2 allele, haemochromatosis gene mutations, and risk for Alzheimer’s disease**

Alzheimer’s disease is a neurodegenerative disease characterised pathologically by the presence of neurofibrillary tangles, senile plaques, and selective loss of neurons. Numerous hypotheses have been suggested for the aetiology and pathogenesis of Alzheimer’s disease and one that has gained considerable

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**Table 1** Frequency of apoE genotypes and E4 alleles in different groups

<table>
<thead>
<tr>
<th>Genotype†</th>
<th>Patients</th>
<th>Non-demented controls</th>
<th>Sporadic FTD</th>
<th>Temporal lobe atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>561</td>
<td>561</td>
<td>98</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>2.3%</td>
<td>2.3%</td>
<td>6.1%</td>
<td>9.7%</td>
</tr>
<tr>
<td>E4/E4</td>
<td>26.0%</td>
<td>26.0%</td>
<td>31.6%</td>
<td>38.7%</td>
</tr>
<tr>
<td>E4/*</td>
<td>71.7%</td>
<td>71.7%</td>
<td>62.3%</td>
<td>61.6%</td>
</tr>
<tr>
<td>E4/0</td>
<td>15.3%</td>
<td>15.3%</td>
<td>21.9%</td>
<td>18.7%</td>
</tr>
<tr>
<td>p-value</td>
<td>0.02</td>
<td>0.004</td>
<td>0.004</td>
<td>0.3</td>
</tr>
</tbody>
</table>

†E4/E4, E4 homozygotes; E4/*, E4 heterozygotes; No E4, all other genotypes.
attention is the disruption of the brain iron metabolism in Alzheimer’s disease that could lead to an oxidative stress and neuronal damage. An increased iron deposition has been found in the Alzheimer’s disease brain, especially in the regions containing more senile plaques and neurofibrillary tangles. Tissue iron can promote oxidative damage through an increase of free radical formation that can lead to subsequent oxidative stress. Among genetic risk factors associated with Alzheimer’s disease, the APOE genotype is the major genetic risk factor for sporadic and familial late onset disease. Recently, two genetic risk factors involved in iron metabolism have been associated with an increased risk for Alzheimer’s disease. The first one is the allele C2 of the transferrin (Tf) gene, an iron transporting protein detected in senile plaques. In another study performed on a small group of patients, mutations in the haemochromatosis associated gene (HFE) were overrepresented in Alzheimer’s disease compared with controls. We postulated that if the genetic defects in iron metabolism were indeed involved in the pathogenesis of Alzheimer’s disease they should be detected in independent populations. Thus, in the present work we evaluated whether the C2 allele of the Tf gene or the two common HFE mutations were involved in the pathogenesis or were a disease modifying factor in our Alzheimer’s disease population.

We included in this study 108 patients with Alzheimer’s disease (80 woman) recruited from both community (n=37) and clinic (n=71) sources. The control sample consisted of 110 unrelated subjects (68 woman) recruited from the community (n=44) and from two clinic sources (n=66). All control subjects underwent a complete neurological and neuropsychological examination to exclude medical illnesses and cognitive impairment. All patients were fully evaluated and met the conventional NINCDS-ADRA criteria for probable Alzheimer’s disease. After informed consent a blood sample was obtained from patients and controls.

The Tf polymorphism (codon 570) was determined after polymerase chain reaction (PCR) amplification using the mismatched sense primer 5′-GCGTGCCCT GTAGTGACC ACCGAGT-3′ and antisense primer 5′-GGAGCCGA AGTGCTCTTACATC-3′ as described. Poly- morphic exon 15 was amplified from genomic DNA using described conditions. The 110 bp product was digested with BstEII, separated in a 6% polyacrylamide gel, and stained with silver nitrate. After digestion the C1 allele was converted to a 89 bp fragment while the C2 allele remained 110 bp long. We also genotyped the two common mutations (H63D and C282Y) involved in hereditary haemochromatosis, APOE genotyping was performed through PCR amplification and HhaI restriction enzyme digestion. Allelic and genotypic distributions were compared by the χ2 test with the SPSS (version 10.0) statistical package.

Mean age for patients and controls was 78.8 (range 61 to 93) and 73.6 years (range 45 to 92) respectively. Both populations were in HWE. The equilibrium for all the polymorphisms. The HFE mutation frequency in the control group was consistent with the frequency of the Spanish population. The frequency we detected of the Tf C2 allele, and C282Y and H63D genotypes among patients with Alzheimer’s disease and controls is given in Table 1. We did not find associations between Tf C2 allele, H63D, and C282Y mutation frequencies and Alzheimer’s disease. Stratification for sex yielded a trend toward an increase in the H63D mutation frequency among male patients with Alzheimer’s disease (53.6%) compared with male controls (33.3%, p<0.009). Stratification for age and APOE status did not yield any significant difference. As expected APOE e4 was increased in the group of patients (47.2%) at least one e4 allele compared with controls (11.8%, p<0.0001).

In this study we did not find any significant association between the Tf C2 allele or the two common mutations in the HFE gene (H63D and C282Y) and Alzheimer’s disease. However, this is by contrast with several studies that have indicated that there is a disruption of brain iron metabolism in Alzheimer’s disease. In neuropathological studies iron has been found to be increased in the brain in Alzheimer’s disease, especially in regions containing abundant neurofibrillary tangles and senile plaques such as the hippocampus and amygdala. In particular, selective accumulation of iron has been found within the neurofibrillary tangles and senile plaques in the Alzheimer’s disease brain. It is of interest that iron is specifically localised to lesional cells surrounding senile plaques, which contain abundant iron binding proteins. Thus, the accumulation of iron in the Alzheimer’s disease brain and the increasing reports implicating oxidative stress, lead us to hypothesise that genetic factors involved in iron metabolism, such as the C2 allele of Tf gene and HFE mutations, could act as a risk factor for the disease. In fact, the C2 allele of the transferrin gene has been associated with an increased risk for Alzheimer’s disease in some studies.

Furthermore, the two mutations of the HFE gene involved in hereditary haemochromatosis, have also been associated with an increased risk for other diseases, such as dilated cardiomyopathy, myocardial infarction, and type 2 diabetes, which are common complications of iron overload. There is only one study assessing HFE mutations in Alzheimer’s disease. In this study, which was performed in 26 patients with familial Alzheimer’s disease, HFE mutations were overrepresented in the group of patients compared with controls.

However, our study is the first assessing HFE mutations in Alzheimer’s disease using a large sample. Based on our results neither the C2 allele of the Tf gene nor the HFE mutations were associated with an increased risk for Alzheimer’s disease. Thus, the effect of the C2 allele of the Tf gene seems to be lower than previously reported. However, our study can not address the influence of these genetic factors on iron deposition. Resolving this point deserves further studies evaluating iron quantification in vivo using MRI or at neuropathological examination.

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References

REM sleep behaviour disorder associated with a neurinoma of the left pontocerebellar angle
REM sleep behaviour disorder (RBD) is a type of parasomnia described by Schenck et al. It is manifested by vigorous body movements, vocalisation, and sometimes injurious behaviour occurring during vivid and violent dreams. Polysomnographic recordings show abnormal abolition of the generalised muscle atonia that occurs during REM sleep, and concurrent bursts of muscle twitching in the
absence of epileptic activity. There is experimental evidence that the area of the brain stem, and especially the pontine tegmentum, is involved in the pathogenesis of the disorder. We report a patient who presented with RBD and was diagnosed and treated for a brain stem neurinoma.

The patient is a 59 year old man, an ex-sailor, who was referred to our clinic because of vivid dreams accompanied by violent behaviour during sleep. He described dreams during which he was trying to defend himself while he was threatened by strangers or attacked by animals. Enacting his dreams, he swore at his “enemies,” and punched and kicked his bed partner. He had repeatedly injured himself crashing into objects or falling out of bed. This aberrant behaviour had been recurring nightly over a period of six years. One year before the onset of his sleep disturbance, he had noticed impaired hearing on the left, which gradually progressed to almost complete left sided deafness.

On admission, neurological examination was unremarkable except for deafness on the left side. Routine laboratory work up— including a full blood count, electrolytes, immunoglobulins, ANA, ds-DNA, and renal, hepatic, and thyroid tests—was normal. Blood glucose was 7.65 mmol/l and serum VDLR was 2+. Brain stem auditory evoked potentials showed a mild delay of waves III–V on the left compared with the right (2.35 ms and 1.96 ms, respectively). The electroencephalogram, including 24 hour EEG monitoring, was within normal limits. Psychiatric and neuropsychological evaluations did not reveal any major psychopathology. Magnetic resonance imaging (MRI) of the brain revealed a 2.3 cm tumour in the left pontocerebellar angle compatible with a neurinoma (fig 1). Cerebrospinal fluid examination showed four white blood cells, an increased protein of 78 mg/dl (normal range 15–45), IgM negative, IgG 4+, VDRL negative, and FTA-Abs 4+. An unexpected finding in our patient was the absence of other prominent neurological signs. There is experimental evidence that the area of the brain stem, and especially the pontine tegmentum, is involved in the pathogenesis of the disorder. We report a patient who presented with RBD and was diagnosed and treated for a brain stem neurinoma.

The RBD was initially treated symptomatically with 1 mg clonazepam at bedtime. This resulted in a remarkable clinical improvement, beginning on the third day of treatment. About three weeks later, the tumour was surgically removed and the diagnosis of neurinoma was confirmed histologically. Following surgery, RBD manifestations completely disappeared. Subsequently, clonazepam was gradually discontinued over a one month period. At six month follow up, the patient reported no aberrant behaviour during sleep.

The syndrome of RBD can be idiopathic or it can be a symptom of various neurological diseases. It usually affects middle aged men. Cases of symptomatic RBD are most often associated with Parkinson's disease, multiple system atrophy, primary dementia, olivopontocerebellar degeneration, and Lewy body dementia. In some of these conditions, RBD may precede other symptoms by years. To our knowledge, there has only been one previous mention of RBD being associated with tumours of the brain stem. Two cases of RBD associated with a neurinoma of the left pontocerebellar angle which obviously caused his typical RBD episodes by interfering with the brain stem neuronal circuitry. As this circuitry extends bilaterally, the lesion must have affected the pontine region on both sides to cause RBD, perhaps through local oedema.

An unexpected finding in our patient was his latent syphilis, which raised the possibility of the aetiological association between the two conditions. To our knowledge, there has only been one previous mention of RBD being associated with tumours of the brain stem. Two cases of RBD associated with a neurinoma of the left pontocerebellar angle which obviously caused his typical RBD episodes by interfering with the brain stem neuronal circuitry. As this circuitry extends bilaterally, the lesion must have affected the pontine region on both sides to cause RBD, perhaps through local oedema. An unexpected finding in our patient was his latent syphilis, which raised the possibility of the aetiological association between the two conditions.

In conclusion, RBD may be symptomatic of an underlying brain stem tumour. Thus clinicians should consider the possibility of structural brain stem lesions whenever aberrant behaviour during sleep is present, even in the absence of other prominent neurological signs. A polysomnographic recording in conjunction with brain imaging studies should be performed to investigate the possibility of the coexistence of a brain tumour and RBD. Should that be the case, neurosurgical treatment is clearly indicated.
REM sleep behaviour disorder associated with a neurinoma of the left pontocerebellar angle

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