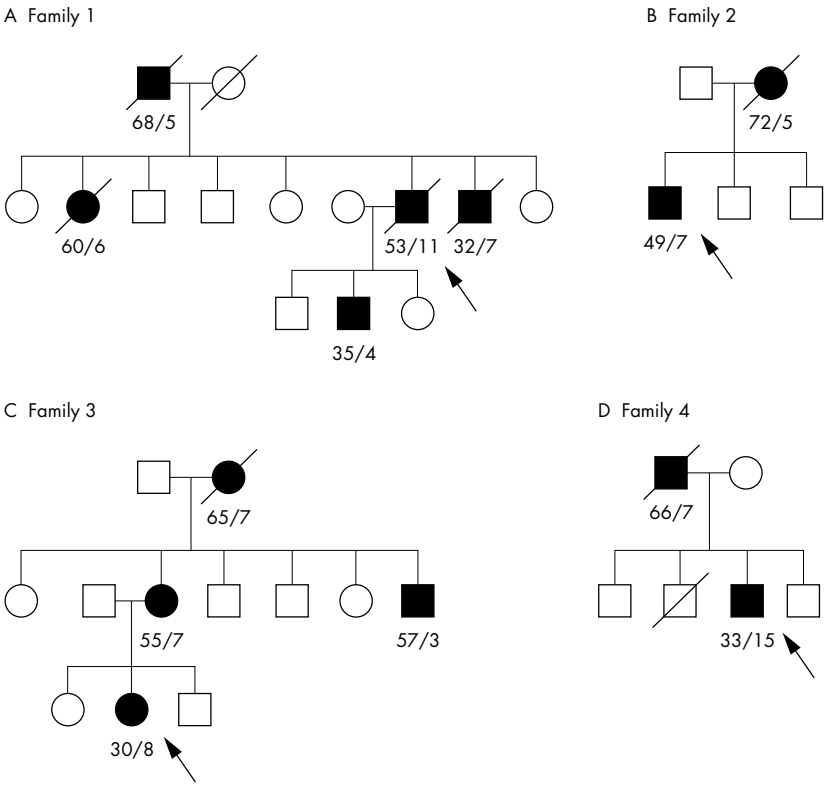
LETTERS

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.[1] 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 missense mutations at codon 84 (L84F and L84V) in the SOD1 mutations in Japan, the United States, and Italy.[2] 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 ($p=0.0005$ 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.[1 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.[3] 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 most of the polyglutamine diseases, familial amyloidotic polyneuropathy (FAP) with V30M mutation of transthyretin and Creutzfelt-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.[4 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[4] and CJD[5], 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.



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

Parent age of onset (y)	64.4 (6.30, 2.10)
Offspring age of onset (y)	44.8 (12.1, 4.06)
Paired difference in age of onset	19.6 (10.4, 3.48)
p Value (paired difference in age of onset)	0.0005†/0.0077‡
Parent duration of disease (y)	6.55 (1.94, 0.64)
Offspring duration of disease (y)	7.55 (3.61, 1.20)
Paired difference in duration	1.00 (4.55, 1.51)
p Value (paired difference in duration)	0.5287†/0.4008‡

Figure1 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[6] (reprinted from *J Neurol Sci* with permission from Elsevier Science), and expressed as mean (SD, SEM). *Paired *t* test; †Wilcoxon test.

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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 in many studies,[1] 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,[2] other studies of cases of FTD with pathological confirmation or tau mutations did not confirm this effect.[3–5] However, recently it has been shown that semantic dementia, the temporal variant of FTD, may be associated with higher frequencies of the apoE4 allele.[6] 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 clinicoepidemiological survey of patients with FTD in The Netherlands.[2] 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 ΔK280), 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 definitely established due to incomplete or inconclusive neuropsychological testing. The remaining 18 patients (58%) showed mainly decreased spontaneous speech and word finding difficulties. 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.[7] All patients and controls were genotyped for the apoE allele as described by Slooter *et al*.[1] Both genotype frequencies and apoE4 allele frequencies were calculated for each group and compared with non-demented controls using a χ^2 test.

Six per cent of the 98 patients with sporadic FTD had the apoE4/E4 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/*) 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.[8] 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. 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 neuroimaging 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.

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

		Genotype†			Alleles	
Group	Patients	E4/E4	E4/*	No E4	%E4	P-value
Non-demented controls	561	2.3%	26.0%	71.7%	15.3	Reference
Sporadic FTD	98	6.1%	31.6%	62.3%	21.9	0.02
Temporal lobe atrophy	31	9.7%	38.7%	51.6%	29.0	0.004
Frontal lobe atrophy	67	4.5%	28.4%	67.1%	18.7	0.3

†E4/E4, E4 homozygotes; E4/*,E4 heterozygotes; No E4, all other genotypes.

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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