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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 neu-

roimaging 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.

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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

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

| Group | Patients | Genotype† | | | Alleles | | P-value |
|-----------------------|----------|-----------|-------|-------|---------|-----------|---------|
| | | E4/E4 | E4/* | No E4 | %E4 | | |
| 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.

attention is the disruption of the brain iron metabolism in Alzheimer's disease that could lead to an oxidative stress and neuronal damage.¹ An increased iron deposition has been found in the Alzheimer's disease brain, especially in the regions containing more senile plaques and neurofibrillary tangles.^{1,2} Tissue iron can promote oxidative damage through an increase of free radical formation that can lead to subsequent oxidative stress.¹ 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.^{3,4} In another study⁵ 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 these 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 investigated 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 clinic sources (n=66). All control subjects underwent a complete neurological and neuropsychological examination to exclude medical illness 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'-GCTGTGCCTT GATGGTACC AGGTAA-3' and antisense primer 5'-GGA CGCA AGCTTCCTTATCT-3' as described.³ Polymorphic exon 15 was amplified from genomic DNA using described conditions.³ The 110 bp product was digested with *Bst*EII, 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.⁵ APOE genotyping was performed through PCR amplification and *Hha*I restriction enzyme digestion. Allelic and genotypic distributions were analysed by the χ^2 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 Hardy-Weinberg equilibrium for all the polymorphisms. The HFE mutation frequency in the control group was consistent with the frequency of the Spanish population. The frequency distribution 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

Table 1 Frequencies of the C2 allele of the Tf gene, and C282Y and H63D mutations among AD patients and controls

| Genotype | AD patients (% (n)) | Controls (% (n)) |
|-------------------|---------------------|------------------|
| Tf gene: | | |
| C2 + | 31.5 (34)* | 35.5 (39) |
| C2 - | 68.5 (74) | 64.5 (71) |
| Allelic frequency | | |
| C1 | 0.82 | 0.81 |
| C2 | 0.17 | 0.18 |
| H63D + | 42.6 (46)† | 34.5 (38) |
| Allelic frequency | 0.26 | 0.20 |
| C282Y + | 3.7 (4)‡ | 3.6 (4) |
| Allelic frequency | 0.02 | 0.02 |

*†‡Not significant.
+, one or two alleles.

an increase in the H63D mutation frequency among male patients with Alzheimer's disease (53.6%) compared with male controls (33.3%, $p=0.09$). Stratification for age or APOE status did not yield any significant difference. As expected APOE $\epsilon 4$ was increased in the group of patients (47.2% at least one $\epsilon 4$ 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.^{1,2} 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.² In particular, selective accumulation of iron has been found within the neurofibrillary tangles and senile plaques in the Alzheimer's disease brain.^{1,2} It is of interest that iron is specifically localised to lesions of Alzheimer's disease and not the glial cells surrounding senile plaques, which contain abundant iron binding proteins.¹ 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.^{3,4} 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 study⁶ 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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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.*^{1,2} 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