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 study[5] 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 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 ϵ4 was increased in the group of patients (47.2% at least one ϵ4 allele) compared with controls (11.8%, p<0.0001).

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.

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 study[5] 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

1 **Smith MA**, Harris PL, Sayre LM, *et al*. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc Natl Acad Sci USA* 1997;**94**:9866–8.
2 **Good PF**, Perl DP, Bierer LM, *et al*. Selective accumulation of aluminum and iron in the neurofibrillary tangles of Alzheimer's disease: a laser microprobe (LAMMA) study. *Ann Neurol* 1992;**31**:286–92.
3 **Namekata K**, Imagawa M, Terashi A, *et al*. Association of transferrin C2 allele with late onset Alzheimer's disease. *Hum Genet* 1997;**101**:126–9.
4 **van Rensburg SJ**, Carstens ME, Potocnik FC, *et al*. Increased frequency of the transferrin C2 subtype in Alzheimer's disease. *Neuroreport* 1993;**4**:1269–71.
5 **Moalem S**, Percy ME, Andrews DF, *et al*. Are hereditary hemochromatosis mutations involved in Alzheimer disease?. *Am J Med Genet* 2000;**93**:58–66.

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

absence of epileptic activity.[1] There is experimental evidence[3 4] that the area of the brain stem, and especially the pontine tegmentum, is involved in the pathogenesis of the disorder.[4] 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 VDRL 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), FTA-Abs 4+, FTA-Abs IgM negative, IgG 4+, VDRL negative, and TPHA positive in a dilution of 1:640. Because the patient had never been treated for syphilis, which presumably had been latent for an unknown period of time, 30 million units of a penicillin G were given daily intravenously for 10 days. A polysomnogram coupled with videotape recording was performed through the night for eight hours to evaluate the patient's sleep disorder. This showed lack of muscle atonia during most REM periods and bursts of muscle twitching of the arms and legs recorded electromyographically, in the absence of epileptic activity. These polysomnographic findings, along with the videotaped body movements, were considered pathognomonic of RBD.

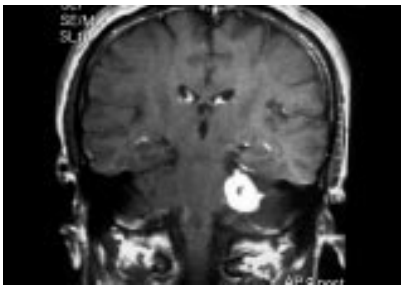

Figure 1 A neurinoma of the left pontocerebellar angle, 2.3 cm in diameter, shown on magnetic resonance imaging of the brain (T2 weighted image).

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 a 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.[2] Cases of symptomatic RBD are most often associated with Parkinson's disease, multiple system atrophy, primary dementia, olivopontocerebellar degeneration, and Lewy body dementia.[2 5] 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.[2] Our patient had 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 an alternative cause for RBD. Syphilis could have affected the brain stem network involved in the pathogenesis of RBD. However, we ruled out this possibility for the following reasons: first, the patient did not present with active infection, as indicated by the relevant serological and CSF findings (negative FTA-Abs IgM antibodies); second, he did not have any obvious residual clinical signs or symptoms of CNS syphilis; third, his RBD had remained relatively stable over the previous six years; moreover, the development of the tumour obviously preceded the occurrence of the abnormal sleep behaviour by at least a year, as evidenced by the presence of impaired hearing since that time; and finally, the complete remission of RBD following surgical removal of the neurinoma and the absence of any relapse during a six month follow up provides direct evidence for 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.

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References

1 **Schenck CH**, Bundlie SR, Hinger MG, *et al.* Chronic behavioral disorders of human REM sleep: a new category of parasomnia. *Sleep* 1986;**9**:293–308.
2 **Schenck CH**, Hurwitz TD, Mahowald MW. REM sleep behavior disorder: an update on a series of 96 patients and a review of the world literature. *J Sleep Res* 1993;**2**:224–31.
3 **Jouvet M**, Delorme F. Locus coeruleus et sommeil paradoxal. *C R Soc Biol* 1965;**159**:895–9. [In French.]
4 **Morrison AR**. Mechanisms underlying oneiric behavior released in REM sleep by pontine lesions in cat. *J Sleep Res* 1993;**2**:4–7.
5 **Turner RS**, D'Amato CJ, Chervin RD, *et al.* The pathology of REM sleep behaviour disorder with comorbid Lewy body dementia. *Neurology* 2000;**55**:1730–2.