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The hemochromatosis gene affects the age of onset of sporadic Alzheimer's disease

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Abstract

NEUROBIOL AGING. In the present study we analysed the genotype of HFE, the gene involved in hemochromatosis, in 107 patients with sporadic late-onset AD and in 99 age-matched non-demented controls. We observed that patients carrying the mutant HFE-H63D allele had a mean age at onset of 71.7 ± 6.0 years versus 76.6 ± 5.8 years of those who were homozygous for the wild-type allele ($p = 0.001$). The frequency of the HFE-H63D mutation was highest (0.22) in the patients aged <70 years at the time of disease onset, whereas it was 0.12 in those with disease onset at an age of 70–80 years, and 0.04 in those aged more than 80 years. The APOE genotype did not significantly modify the effect of HFE on age at onset. We conclude that mild disturbances of iron homeostasis associated with a common genetic determinant may interact with other pathogenic mechanisms involved in AD. HFE mutations may anticipate AD clinical presentation in susceptible individuals.

Keywords: Alzheimer's disease; Dementia; Hemochromatosis; Iron; APOE; Mutation; Disease onset

1. Introduction

Various lines of evidence suggest that Alzheimer's disease (AD) is not a single disease but a clinical syndrome due to different genetic and environmental factors leading to a relatively uniform clinical and histopathological appearance [35]. The recognised genetic factors include mutations of the genes encoding the amyloid precursor protein [14] and presenilin 1 and 2 [16,31], which are responsible for a small proportion of familial and usually early-onset AD cases. A fourth gene encoding apolipoprotein E (APOE) is also implicated in the risk of developing the disease [4,23,29,36]. However, although APOE-$\epsilon 4$ is a major risk factor for AD, it is neither necessary nor sufficient for the development of the disease and the presence of other genetic or acquired factors has been postulated.

In vitro and pathological observations have led to the hypothesis that iron may play a role in the pathogenesis of AD, possibly through free-radical production [33,34]. The "iron hypothesis" is further supported by the presence of iron in amyloid plaques and neurofibrillary tangles [33], and the demonstration that iron facilitates the aggregation of β-amyloid peptide [7,17] and increases β-amyloid toxicity [30]. Increased amounts of loosely bound iron have been detected in brain samples taken from AD patients [15]. The discovery of HFE [12], a protein that plays a key role in the regulation of body iron, and of HFE gene mutations causing genetic hemochromatosis has opened up new perspectives in the study of the relationship between iron and AD. The known function of HFE is to complex the transferrin receptor on the cell membrane and lowering its affinity for iron-bound transferrin [13]. Hemochromatosis is the most common inherited monogenic disorder in people of European descent. It is characterised by an inappropriately increased absorption of dietary iron that leads to excess iron deposition in various tissues and organs [24]; it is inherited as an autosomal recessive trait linked to the major histocompatibility complex on the short arm of chromosome 6. Although severe iron overload is typical of homozygous hemochromatosis, minor modifications of serum iron, transferrin-saturation and serum ferritin can also be observed in a fraction of heterozygotes, but very rarely they give rise to complications [6]. However, the mild modifications of iron status induced by heterozygous hemochromatosis can influ-

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ence the severity and clinical evolution of heterogeneous conditions such as hepatitis [32], porphyria cutanea tarda [27] and cardiovascular disease [26]. Two common missense HFE gene mutations have been associated with hemochromatosis: a large proportion of patients with severe hemochromatosis are homozygous for the major C282Y mutation, which is very frequent in populations of Celtic ancestry but less frequent in Mediterranean countries [19]; the second common mutation is H63D, which has a less evident effect on iron status but an increased frequency on the chromosomes of hemochromatosis patients not bearing the C282Y [12]. The H63D mutation is thought to be more ancient than C282Y and has a high frequency in many populations [19]. Overall, 2–20% of individuals of European descent may have genetically determined mild or latent iron status alterations associated with C282Y, while a much larger proportion carry H63D [11].

A recent study reported an increased frequency of HFE mutations in male patients with familial AD and suggested that HFE mutations could be predisposing for familial AD in males APOE-ε4 negative [20]. The aim of the present study was to investigate whether HFE gene mutations are associated with an increased risk for sporadic AD, and to evaluate their influence on the age of disease onset.

2. Patients and methods

2.1. Patients

Two hundred and six elderly subjects living in the urban area of Milan were recruited at the Day Hospital of the Department of Geriatrics of IRCCS Ospedale Maggiore (Milan, Italy), 107 of whom (35 males, 72 females: age 66–93 years, mean 80 ± 6 years) had a clinical diagnosis of sporadic late-onset (>60 years) Alzheimer's disease. The diagnosis of AD was based on the NINCDS-ADRDA [18] and DSM IV criteria [1]. The results of routine laboratory tests for serum TSH, FT4, B12 vitamin and folates, transferrin saturation and serum ferritin were also obtained. All of the patients underwent brain CT or NMR imaging. The remaining 99 subjects (48 males, 51 females: age 62–92 years, mean 79 ± 7 years) were investigated as controls in order to establish APOE and HFE allele frequencies in an age-matched population. Informed consent was obtained from all of the studied subjects or their relatives.

2.2. Methods

Genomic DNA was extracted from peripheral leukocytes by means of standard procedures. The two HFE mutations were detected by polymerase chain reaction (PCR) DNA amplification of the relevant exons and restriction with Rsal for C282Y and Bcll/Mbol for H63D. The APOE genotypes were determined by means of PCR amplification of a 234 base-pair fragment of exon 4 of the APOE gene followed by digestion with Cfol. The restriction patterns were obtained by means of gel electrophoresis.

The data were analysed using the statistical package Statview 5.0 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

Table 1
Frequencies of alleles at the APOE (ε2, ε3, ε4) and HFE loci in 107 patients with sporadic late onset Alzheimer's disease and in 99 control subjects. Y282 and D63 identify the two common mutations associated with genetic hemochromatosis.

	Alzheimer's D. (214 alleles)	Controls (198 alleles)	
APOE			P*
ε2	0.03	0.04	
ε3	0.73	0.87	0.0006
ε4	0.24	0.09	
HFE			
Y282 (mutant)	0.02	0.02	1.00
C282 (wild type)	0.98	0.98	
D63 (mutant)	0.11	0.14	0.46
H63 (wild type)	0.89	0.86	

* (χ^2 test)

3. Results

The allelic frequencies of APOE and HFE alleles in the AD patients and controls are shown in Table 1. Five homozygotes and 41 heterozygotes for the APOE-ε4 allele were found among the AD patients, and two homozygotes and 15 heterozygotes among the controls. The HFE-C282Y mutation was found in the heterozygous state in four AD patients and four controls; given the low frequency of this mutation, it was not further analysed. The H63D allele was common in both patients and controls, being present in 22 AD patients (two homozygotes) and 25 controls (one homozygote). The observed genotype distribution for APOE and HFE genes was not significantly different from that expected by the Hardy-Weinberg equilibrium. We also compared the distribution of the combined genotypes at the two loci with the expected frequencies calculated for each group from allelic frequencies, and no preferential allelic association was found. The frequency of the APOE-ε4/H63D association in AD patients was marginally greater than expected, but this difference was not statistically significant. No difference in the distribution of APOE and HFE genotypes was observed between male and female patients.

When the age of onset of AD in the presence or in the absence of APOE-ε4 was compared, it was found that the 46 patients carrying one or two APOE-ε4 alleles had a non-significant earlier onset than those with the other ApoE genotypes (74.7 ± 5.9 vs. 76.3 ± 6.3 years: p = 0.19, Mann-Whitney U-test). The patients who were heterozygous or homozygous for the HFE-H63D allele experienced an earlier disease onset than those who were homozygous

Table 2
Allelic frequencies of APOE and HFE alleles in 107 Alzheimer's disease patients stratified by age at onset (A) and in 99 control subjects stratified by age (B).

A) Age at onset (years)	Number of patients	Allele frequency of APOE-ϵ4	Allele frequency of HFE-H63D
60–70	23	0.26	0.22*
70–80	47	0.28	0.12
>80	37	0.16	0.04*

* (P = 0.004, Fisher's exact test)

B) Age (years)	Number of subjects	Allele frequency of APOE-ϵ4	Allele frequency of HFE-H63D
60–70	15	0.10	0.16
70–80	26	0.13	0.13
>80	58	0.06	0.13

for the wild allele (71.7 ± 6.0 vs. 76.6 ± 5.8 years: p = 0.001, Mann-Whitney U-test); this effect was similar in males and females. Table 2 shows the allelic frequencies of APOE and HFE in three groups of AD patients stratified by age at onset: the HFE mutation was more frequent (0.22) in the patients with disease onset at an age <70 years; this was almost twice the frequency observed in those aged 70–80 years at onset (0.12) and five times more than that observed in the patients aged more than 80 years (0.04). The frequency of the APOE-ϵ4 allele was not significantly less in the patients with a later disease onset. No significant age-related difference in HFE allelic frequency was observed in controls (Table 2), but there was a trend towards a lower frequency of APOE-ϵ4 in those aged more than 80 years. There was no significant evidence of an additive effect of the APOE and HFE genotypes on age at disease onset, although the oldest mean age at onset (77.3 years) was observed in the subset of 51 patients who were homozygous for wild-type HFE and did not carry the APOE-ϵ4 variant. Results of Kaplan-Meier analysis of age at onset in relation to HFE and APOE genotypes are shown in Fig. 1: mutant HFE genotypes gave rise to significantly different curves

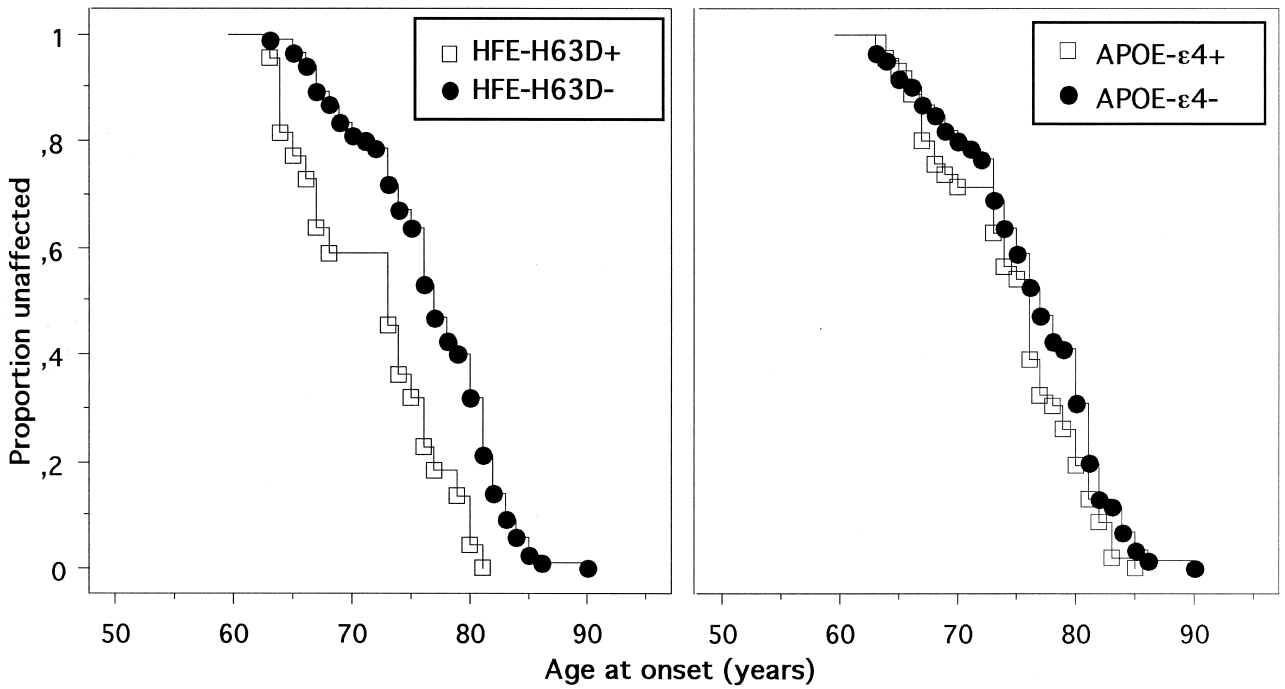

Fig. 1. Kaplan Meier analysis of age at disease onset in 107 individuals with late-onset Alzheimer's disease sorted by APOE and HFE genotypes. In the graph on the left HFE-H63D+ denotes individuals heterozygous or homozygous for the mutation. In the graph on the right APOE-ϵ4+ denotes individuals carrying the ϵ4 allele in the heterozygous or homozygous state; APOE-ϵ4− denotes individuals with genotypes not including ϵ4.

($p = 0.0002$, Mantel-Cox logrank test), whereas the APOE genotypes including the APOE-ϵ4 allele generated a curve that is not significantly different from that of individuals carrying the other APOE alleles.

As evaluated by means of transferrin saturation and serum ferritin, the iron status of patients carrying HFE mutations was not significantly different from that of those with a wild-type HFE genotype (data not shown). In particular, none of the patients carrying HFE mutations showed signs of iron overload.

4. Discussion

The possible role of iron in the pathogenesis of some neurodegenerative disorders prompted us to evaluate hemochromatosis mutations in subjects with AD. To our knowledge this is the first study to examine the distribution of HFE mutations in sporadic late-onset AD typed for APOE, and to analyse the effect of HFE on the age of presentation. The principal HFE-C282Y hemochromatosis mutation is less frequent in Italy than in Northern Europe [8], and was poorly represented in our patient and control groups. We therefore concentrated on analysing the H63D mutation which is less affected by ethnic background, having a world wide allelic frequency of 0.08 and being present in all European populations at allele frequencies greater than 0.06 [19].

The main finding of this study was the effect of a HFE mutation on the age of presentation of AD. The age at onset of patients carrying one or two copies of H63D was an average of five years less than that of patients with a wild-type HFE genotype. Although the overall frequency of H63D was similar in patients and controls, its distribution was different in patient groups stratified by age at disease onset, being five times more frequent in patients manifesting the disease at an age <70 years as compared with those developing the disease after the age of 80 years. This difference did not appear to be related to the chronological age of the patients or to an effect on survival independent from AD, since the allelic frequency of H63D did not vary in age-stratified groups of controls. On the contrary, the overall frequency of APOE-ϵ4 was higher in AD patients than in controls, but there was a less marked modification with increasing age, with a trend towards a reduction being observed only in the subset of patients with an age at disease onset of more than 80 years. Since APOE-ϵ4 is also a risk factor for cardiovascular death [10,37], the possibility should be considered that its reduced presence in the oldest group may have been due to a survival effect, since it was also lower in the control subjects aged more than 80 years than in those who were younger. We also explored the possibility of an interaction between APOE and HFE, but did not find any significant evidence that the two loci exert a synergistic effect. However, the fact that the oldest age at onset was observed in the patients without either the APOE-ϵ4 or the HFE-H63D mutation may provide indirect evidence in support of such effect.

The interpretation of these data is not univocal. Our findings suggest that the mutation of a gene responsible for the regulation of the iron status may interact with other mechanisms involved in AD, affecting the disease progression in the preclinical stage, and leading to an anticipation of clinical diagnosis. The design of our study does not allow to assess whether HFE mutations affect the overall risk of susceptible individuals developing AD, or to demonstrate that the rate of disease progression after clinical onset is related to HFE genotypes. Additional studies of affected and unaffected individuals from AD pedigrees, as well as longitudinal observations, are needed in order to clarify these aspects. Since the C282Y mutation is common in populations of Northern-Europe, further studies in those populations will be necessary to establish whether the effect of H63D on the age of onset of AD is shared (and possibly amplified) by the C282Y mutation. Finally, it is interesting to note that a relationship between the major histocompatibility complex and the age of presentation of AD has been proposed in the past. It was observed that the HLA-A2 allele was associated with an anticipation of AD onset [2,22]. Because HLA and HFE are closely linked genetic loci, the HLA-A2 allele is included in haplotypes in linkage disequilibrium with HFE mutant alleles [11].

The first study to suggest a relationship between hemochromatosis and AD [20] analysed HFE and APOE genotypes in a group of patients with familial AD. A complex interaction between the two loci, different in males and females, was suggested by the Authors: among APOE-ϵ4 negative males the absence of HFE mutations would be protective against AD, while among APOE-ϵ4 negative females the presence of HFE mutations would afford some protection against AD. The relatively small number of cases included in that study, however, did not allow an extensive comparison of all APOE/HFE genotypes between patients and controls, and age at onset was not reported. Our data are in agreement with the cited study in showing an overrepresentation of HFE mutations in patients with an earlier disease onset. We failed however to observe any gender-related difference. The different selection of AD patients, familial versus sporadic, could account, at least in part, for the different findings. Patients with familial AD are usually younger as compared with those with the sporadic disease: as a consequence the iron status of women in the two groups could have been different. In our series the iron status of the individuals carrying HFE mutations was not significantly altered or different from that of the subjects with wild-type HFE genotypes, but it is possible that subtle alterations in iron metabolism may not be reflected by standard iron parameters.

A possible link between HFE and iron-related damage in the brain is provided by the analysis of HFE expression in different organs and tissues. The immunohistochemical staining pattern of HFE in human tissues has been defined

as a subset of transferrin receptor-positive cells [3]. Surprisingly, the capillary endothelium of the brain seems to be one of the major sites of HFE expression and scattered cortical cells (possibly astrocytes—the same cell type that produces ApoE [5]) also stain for HFE [3]. Of the endothelial cells examined, apart from the liver, HFE was only found in the vascular endothelium of the brain, thus indicating the importance of its role in limiting the movement of iron across the blood-brain barrier. The expression of HFE by glial cells makes it possible to speculate that the presence of mutant HFE species may contribute towards iron accumulation in these cells and/or its leakage into the surrounding extracellular space, thus increasing the availability of the free-iron that appears to play a relevant role in the evolution of β-amyloid plaques [25].

HFE is not the first gene encoding for an iron related protein to be involved in AD. The TfC2 allelic variant of transferrin has been reported to have an increased frequency in late-onset AD [21]. The finding was confirmed in several populations, and it was hypothesised that defective binding of iron and aluminium by TfC2 could be responsible for free radical damage contributing to the pathological lesions of AD [38]. Further studies are warranted to investigate the relative role and reciprocal influence of HFE genotypes and of transferrin variants in AD.

Protection against oxidative damage has already been demonstrated to slow the clinical progression of AD [28], and chelation therapy with the iron chelator desferrioxamine was able to reduce the disease progression in patients with sporadic AD [9]. The identification of this novel AD-related genetic determinant provides new insights into the pathogenesis of the disease, and may also contribute towards the diagnosis and treatment of individuals from families at risk of AD carrying HFE mutations.

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