ELECTRONIC LETTER

Iron genes, iron load and risk of Alzheimer's disease

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Background: Compound heterozygotes of the haemochromatosis gene (*HFE*) variants, H63D and C282Y, have raised transferrin saturation compared with that in the wild type. In the cohort of the Oxford Project To Investigate Memory and Ageing (OPTIMA), bicarriers of the *HFE* C282Y and the transferrin C2 gene variants are at five times greater risk of developing Alzheimer's disease; the addition of *HFE* H63D may raise the risk still further.
Objective: To investigate transferrin saturation by *HFE* and transferrin genotype among people without dementia–that is, controls and those with mild cognitive impairment (MCI)—and also among those with Alzheimer's disease.
Methods: Serum iron status and genotype were examined of 177 patients with Alzheimer's disease, 69 patients with MCI and 197 controls from the OPTIMA cohort.
Results: Although each of these variants alone had relatively little effect on iron status, the combination of either *HFE* C282Y and *HFE* H63D or of *HFE* C282Y and transferrin C2 markedly raised transferrin saturation in those without dementia, but had little effect in those with mature Alzheimer's disease.
Conclusions: These combinations may raise the risk for Alzheimer's disease, owing to higher iron loads and therefore oxidative stress in the preclinical phase. If replicated, these findings will have implications for the prevention of Alzheimer's disease.

Key points

- Bicarriers of the haemochromatosis gene (*HFE*) C282Y and the transferrin C2 variants may have higher iron loads than those of the wild type, as has previously been shown for compound heterozygotes of *HFE* H63D and *HFE* C282Y.
- The association of the combination of *HFE* C282Y and transferrin C2 with increased risk for Alzheimer's disease, as previously reported, may therefore be due to oxidative stress in the preclinical phase.
- If confirmed, these results have implications for the prevention of Alzheimer's disease in the 6% of northern Europeans who carry one or both combinations, as iron overload is a treatable condition.

The effects of the haemochromatosis gene (*HFE*) variants, H63D and C282Y, on serum iron status of a healthy human population were first examined in 1998[1] and have been extensively studied since. Several large studies[2–6] have obtained broadly consistent results. For instance, Jackson *et al*[3] summarised their results for transferrin saturation as follows: C282Y homozygotes >compound heterozygotes (C282Y and H63D) >H63D homozygotes >C282Y heterozygotes >H63D heterozygotes >wild type. However, to our knowledge, the influence on iron status of the combination of *HFE* and transferrin variants has not been studied, either in healthy controls or in those with Alzheimer's disease.

We previously reported[7] that in the cohort of the Oxford Project To Investigate Memory and Ageing (OPTIMA), carriers of either the *HFE* C282Y or transferrin C2 variants alone had no effect on risk for Alzheimer's disease; however, bicarriers of the two alleles, whether heterozygotes or homozygotes, were at five times greater risk for Alzheimer's disease. The addition of the third variant, *HFE* H63D, seemed to increase the risk still further.

Alzheimer's disease has a long preclinical phase.[8] One of the early events in the development of the disease is thought to be oxidative stress,[9 10] due crucially to the actions of redox-active iron.[11 12] The above results therefore suggested that combinations of the *HFE* C282Y, *HFE* H63D and transferrin C2 alleles might contribute to higher iron loads during the preclinical phase of Alzheimer's disease. We therefore investigated transferrin saturation by genotype among those without dementia—that is, controls and patients with mild cognitive impairment (MCI)—as well as among patients with Alzheimer's disease, in the OPTIMA cohort. We examined combinations of the *HFE* H63D, *HFE* C282Y and transferrin C2 alleles.

METHODS

All 177 patients with Alzheimer's disease (100 women), 69 with MCI (30 women) and 197 controls (98 women) were Caucasians from the longitudinal, observational cohort of OPTIMA, drawn from the Oxford region. Mean age at onset of Alzheimer's disease was 70.3 (standard deviation (SD) 9.1) years, and age at death or last examination of controls was 76.2 (9.7) years (195 controls). Of the patients with Alzheimer's disease, 112 were neuropathologically confirmed by the criteria of the Consortium to Establish a Registry for Alzheimer's Disease[13] (99 had ''definite'' and 13 had ''probable'' disease) and 65 were diagnosed as having ''probable Alzheimer's disease'' by NINCDS-ADRDA criteria.[14] Possible autosomal dominant cases were excluded on the basis of family history. Diagnosis of MCI was according to Petersen *et al*.[15] All 197 controls were without cognitive impairment and had Cambridge Cognitive Examination scores[16] >80. Informed consent was obtained in writing from all participants and the study was approved by the Central Oxford Ethics Committee (approval number 1656).

Blood samples were taken during the earlier assessments, when people with MCI were still controls—that is, between December 1989 and March 2003. Samples were then stored at −70℃ until the iron assays were carried out between

Abbreviations: *APOE4*, apolipoprotein E ϵ4 allele; *HFE*, haemochromatosis gene; MCI, mild cognitive impairment; OPTIMA, Oxford Project To Investigate Memory and Ageing

November 2003 and July 2004, during which period they were stored at −20°C. The final diagnoses were made in April 2004. Genotyping of *HFE* C282Y and *HFE* H63D and of transferrin C2 was as described previously.[7] Serum iron and unsaturated iron-binding capacity were determined by a microtitre plate assay[17]; transferrin saturation was calculated from values for serum iron and unsaturated iron-binding capacity.[17]

We used analysis of covariance, followed if significant by pairwise comparisons. A Bonferroni correction factor of 6 was applied to the pairwise comparisons. We used linear regression analysis to test for interactions between variants. In all analyses, we controlled for age, sex and the apolipoprotein E ε4 allele (*APOE*4), removing covariates with $p>0.2$ in steps. All p values are after controlling for these covariates; all mean values are unadjusted.

RESULTS

HFE C282Y and *HFE* H63D

Table 1 shows that with these relatively small numbers, the presence of only one variant allele—that is, *HFE* C282Y or *HFE* H63D—had little effect on transferrin saturation. On the other hand, compound heterozygotes had markedly higher transferrin saturation among controls or when combining controls and those with MCI (hereafter called the non-AD group). We found no significant differences between subgroups of patients with Alzheimer's disease ($p = 0.18$). As we also found no significant differences in any analysis between the first three genetic subgroups—that is, participants negative for either or both variants—we combined these three categories. We found that among the non-AD group, compound heterozygotes had higher transferrin saturation than all others combined: 52.1% (SD 6.0%), *v* 28.7% (11.4%; $p<0.001$), controlling for age, sex and *APOE*4). Also, among the non-AD group, compound heterozygotes were more likely to have iron overload, as measured by transferrin saturation >45% in women or >50% in men, with an adjusted odds ratio (OR) of 8.4 (95% confidence interval (CI) 1.2 to 61). In linear regression analysis, controlling for age, sex and *APOE*4, the interaction between the presence of *HFE* C282Y and *HFE* H63D was a significant predictor of transferrin saturation ($p = 0.008$).

Among compound heterozygotes, patients with Alzheimer's disease had lower transferrin saturation than that in the non-AD group ($p = 0.01$). In contrast, we found no overall reduction in transferrin saturation in patients with Alzheimer's disease.

HFE C282Y and transferrin C2

Table 2 shows the effect of the two variant alleles, *HFE* C282Y and transferrin C2. Again, the presence of only one variant had little effect on transferrin saturation. Only bicarriers of the two variants, whether heterozygotes or homozygotes, had higher transferrin saturation and only among the non-AD group. We found no significant differences between subgroups of patients with Alzheimer's disease ($p = 0.81$). Again, we combined the first three genetic subgroups, as we found no significant differences in any analysis between them. Among the non-AD group, bicarriers had higher transferrin saturation than all others combined: 41.4% (SD 9.7%), versus 28.8% (SD 11.6%; $p = 0.007$, controlling for age, sex and *APOE*4). Also among the non-AD group, bicarriers, like compound heterozygotes, were more likely to have iron overload, with an adjusted OR of 8.3 (95% CI 1.8 to 39). However, in linear regression analysis, controlling for age, sex and *APOE*4, the interaction between *HFE* C282Y and transferrin C2 was not a significant predictor of transferrin saturation ($p = 0.13$).

Among bicarriers, as among compound heterozygotes, patients with Alzheimer's disease had lower transferrin saturation than non-AD ($p = 0.004$).

HFE H63D and transferrin C2

We found no significant differences in transferrin saturation owing to these two variants occurring either singly or in combination.

HFE C282Y, *HFE* H63D and transferrin C2

This combination of all three variants was not examined, as we had only five patients with this combination: four with Alzheimer's disease, one with MCI and no controls.

DISCUSSION

In much larger studies,[2 3 5 6] even single copies of either *HFE* C282Y or *HFE* H63D produce small, significant differences in iron status. However, examination of our present results with those of our earlier study[7] suggests that a combination of two variants is needed to produce a substantially higher iron

Table 1 Transferrin saturation (%) in controls, in those with mild cognitive impairment and in those with Alzheimer's disease, by haemochromatosis genotype

Subgroup	Controls	MCI	All non-AD	AD
HFE C282Y negative, *HFE* H63D negative	28.5 (11.6), 115	27.7 (11.8), 37	28.3 (11.6), 152	28.5 (13.6), 109
HFE C282Y negative, *HFE* H63D positive	30.1 (12.2), 61	23.7 (7.8), 15	28.9 (11.7), 76	31.4 (16.5), 43
HFE C282Y positive, *HFE* H63D negative	30.9 (8.0), 17	30.3 (10.5), 16	30.6 (9.2), 33	27.1 (8.0), 19
HFE C282Y positive, *HFE* H63D positive	52.3 (6.9), 4	51.4 (-), 1	52.1 (6.0), 5	39.2 (6.2), 6
All	29.7 (11.9), 197	27.8 (11.1), 69	29.2 (11.7), 266	29.4 (13.9), 177
p (ANCOVA)*	0.006	0.053	0.001	0.18

Values are mean (SD), n.
AD, Alzheimer's disease; ANCOVA, analysis of covariance; *HFE*, haemochromatosis gene; MCI, mild cognitive impairment; non-AD, controls and those with MCI.
*p Values are after controlling for age, gender and *APOE4* status; mean values are unadjusted. Pairwise comparisons, with Bonferroni correction, compound heterozygotes versus each other subgroup in the above order; in controls: $p=0.004$, $p=0.01$, $p=0.049$; in the non-AD group: $p<0.001$, $p=0.001$, $p=0.004$.

Table 2 Transferrin saturation (%) in controls, in those with mild cognitive impairment and in those with Alzheimer's disease, by haemochromatosis and transferrin genotype

Subgroup	Controls	MCI	All non-AD	AD
HFE C282Y negative, *TF* C2 negative	28.9 (12.2), 102	26.8 (11.5), 37	28.4 (12.0), 139	29.5 (15.1), 85
HFE C282Y negative, *TF* C2 positive	29.2 (11.3), 74	26.0 (9.9), 14	28.7 (11.1), 88	29.2 (14.4), 61
HFE C282Y positive, *TF* C2 negative	33.9 (12.4), 17	27.3 (8.3), 12	31.2 (11.2), 29	32.5 (9.2), 10
HFE C282Y positive, *TF* C2 positive	39.7 (4.8), 4	43.0 (13.7), 4	41.4 (9.7), 8	28.3 (9.1), 15
All	29.7 (11.9), 197	27.7 (11.3), 67	29.2 (11.7), 264	29.5 (14.1), 171
p (ANCOVA)*	0.14	0.10	0.03	0.81

Values are mean (SD), n.
AD, Alzheimer's disease; ANCOVA, analysis of covariance; *HFE*, haemochromatosis gene; MCI, mild cognitive impairment; non-AD, controls and those with MCI; *TF*, the transferrin gene.
*p Values are after controlling for age, sex and *APOE4* status; mean values are unadjusted. Pairwise comparison, with Bonferroni correction, bicarriers versus those with neither variant, in the non-AD group: p = 0.03.

load—that is, sufficient to have a marked effect on the risk for Alzheimer's disease.[7] That combination could be either *HFE* C282Y and *HFE* H63D, or *HFE* C282Y and transferrin C2. It seems that *HFE* C282Y is an essential element in these combinations, as the other two variants together but without *HFE* C282Y had little effect either on the risk for Alzheimer's disease[7] or on iron status. Feder *et al*[18] suggested that, although both the HFE C282Y and the HFE H63D proteins are dysfunctional, HFE C282Y may be more so; any dysfunction associated with the transferrin C2 protein is yet to be established.[19]

The damaging effects of the at-risk combinations were limited to those without dementia and were not seen in those with fully developed Alzheimer's disease. This is consistent with the view that misregulated iron and the associated oxidative stress exert their harmful influence in the preclinical phase of Alzheimer's disease. Some other risk factors for Alzheimer's disease, such as high blood pressure,[20 21] are mainly or only seen in the presymptomatic phase. The lack of any raised iron status associated with the at-risk combinations in patients with mature Alzheimer's disease could be due to iron withholding (anaemia of inflammation), as Alzheimer's disease is a chronic inflammatory condition. However, we found no evidence of iron withholding in our cohort with Alzheimer's disease (data not shown), other than among these two genetic combinations.

In our earlier study,[7] we found that the presence of *APOE*4 further increased the risk for Alzheimer's disease associated with *HFE* C282Y and transferrin C2. In this study, we found no effect of *APOE*4 on transferrin saturation (data not shown). This suggests that *APOE*4 does not contribute directly to iron load, but rather aggravates the oxidative stress caused by raised iron levels.[22 23]

Several studies[2–6] have shown a difference between the sexes in the effect of compound heterozygotes on iron status. Our sample size was insufficient to examine this. A general limitation of the present study was the few patients with at-risk combinations, particularly among controls. For instance, the status of bicarriers of *HFE* C282Y and transferrin C2 was seen in only 4 of 197 controls, 4 of 67 patients with MCI and 15 of 172 patients with Alzheimer's disease. These figures illustrate the high risk for Alzheimer's disease associated with bicarrier status (OR 4.6; 95% CI 1.5 to 14). Owing to this shortage of at-risk combinations, and also to capture as many preclinical cases as possible, we combined controls and MCI to form one non-AD group (we found no significant differences in transferrin saturation between controls and those with MCI among bicarriers, among compound heterozygotes or among the other groups combined; also, virtually all those with MCI were controls when their blood was taken). Given the small numbers, it was surprising to achieve as many significant results, for instance, when comparing only eight bicarriers without Alzheimer's disease, first with the 256 others from the non-AD group and then with 15 bicarriers with Alzheimer's disease. Further, the two different genetic combinations had a similar pattern of results (tables 1 and 2).

In conclusion, although the association of *HFE* compound heterozygotes with higher transferrin saturation is well established,[2–6] our study suggests two new findings: that a similar association may exist in bicarriers of *HFE* C282Y and transferrin C2, and that neither association may be found in patients with mature Alzheimer's disease, only in those without dementia. We may thus suggest a mechanism for the associations with risk for Alzheimer's disease reported in our earlier study[7]—higher iron load and therefore oxidative stress in the preclinical phase of Alzheimer's disease. However, our results should be considered provisional. Two larger studies are now needed—one to examine the associations of compound heterozygosity and of bicarrier status on risk for Alzheimer's disease, and another to study the effects of these two genetic combinations on the iron status of those without dementia, particularly elderly people. A further study should examine the genetic effects on iron levels in the brain, both in those with Alzheimer's disease and in those without dementia, using the latest scanning techniques. If replicated, these results will be relevant to the prevention of Alzheimer's disease. The allelic frequencies of our controls were 5.6% for *HFE* C282Y, 17.5% for *HFE* H63D and 24.6% for transferrin C2. Taking more conservative figures of 6%, 13%[3 24] and 19%,[7 25] respectively, from the literature, then 4% of northern Europeans are expected to be bicarriers of *HFE* C282Y and transferrin C2, and just <3% to be compound heterozygotes with both *HFE* C282Y and *HFE* H63D. That makes altogether around 6% of northern Europeans at risk. Further, iron overload is a readily treatable condition.

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