The Association Between H63D Mutations in HFE and Amyotrophic Lateral Sclerosis in a Dutch Population

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Background: Mutations in HFE, a gene defect that can disrupt iron metabolism, have been implicated in increasing the risk of developing amyotrophic lateral sclerosis (ALS).

Objective: To further establish the association between ALS and HFE mutations by investigating whether HFE mutations are associated with an increased risk of developing ALS in a population in the Netherlands and by pooling our results with those from previous studies.

Design: Retrospective study.

Setting: Tertiary referral center for neuromuscular disorders.

Participants: Genotyping for 2 common HFE mutations was performed in 289 patients with ALS and 5886 population-based controls in the Netherlands between January 1, 2000, and December 31, 2004.

Main Outcome Measures: Development of ALS and clinical phenotype were compared among the different HFE genotypes, adjusting for known prognostic factors such as age at onset and sex.

Results: Homozygosity for H63D was associated with an increased risk of developing ALS (odds ratio [OR], 2.2; 95% confidence interval [CI], 1.1-4.1). After pooling our results with those from previous studies, a positive association between H63D homozygotes (OR, 2.7; 95% CI, 1.7-4.4), heterozygotes (OR, 1.5; 95% CI, 1.0-2.1), and mutation carriers (OR, 1.7; 95% CI, 1.1-2.5) was found. Within the patient group, heterozygosity for the H63D mutation was associated with a higher age at onset.

Conclusions: These findings suggest that H63D mutations in HFE play a role in the pathogenesis of ALS in various populations. This association might involve a later-onset subset of ALS.

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out populations, we investigated a large Dutch population for \textit{HFE} mutations and pooled these results with data from previous studies. Because ALS is a heterogeneous disease, we also studied the effect on clinical phenotype (age at onset, bulbar or spinal onset, and survival) to determine whether a particular subset of ALS is associated with \textit{HFE} mutations.

**METHODS**

**PATIENTS**

Between January 1, 2000, and December 31, 2004, 289 patients newly diagnosed as having SALS at the University Medical Center Utrecht, a tertiary referral clinic in the Netherlands, were recruited. Diagnosis was made according to the El Escorial criteria after exclusion of other conditions. Patients with possible, probable, or definite SALS were included. All patients were white. Demographic features, age at onset, site at onset of disease, and survival were recorded. Onset of disease was defined as onset of first weakness, dysarthria, or dysphagia. Survival, as a measure of rate of progression, was defined as the interval between age at onset and age at death from any cause, tracheostomy, or persistent (24 hours a day) ventilatory assistance. The study protocol was approved by the institutional ethical committee of the University Medical Center Utrecht.

**CONTROLS**

Controls were included from 2 prospective studies in the Netherlands described elsewhere. Briefly, from the Rotterdam Cohort Study, a population-based study of 7983 individuals 55 years and older, a random sample of 4275 individuals was genotyped for \textit{HFE} mutations. The other sample was taken from a Dutch contribution to the European Prospective Investigation into Cancer and Nutrition. From this cohort of 17 357 women who attended the regional population-based breast cancer screening program, 1611 women were randomly genotyped for \textit{HFE} mutations.

**GENOTYPING**

After extraction of genomic DNA from whole blood of patients with ALS, mutation analyses for the \textit{C282Y} and \textit{H63D} alleles were performed by an allelic discrimination assay (TaqMan) on an ABI Prism 7000 Sequence Detection System (Ap-
STATISTICAL ANALYSIS

An association between HFE mutations and risk of developing ALS was evaluated with logistic regression analysis, adjusting for the potential confounders age (at onset) and sex. To determine whether HFE mutations are associated with the clinical phenotype, their effect was studied by multivariate regression, adjusting for possible confounders. The influence of HFE mutations on clinical phenotype was analyzed by (1) a linear regression model with age at onset as the outcome variable, adjusting for sex and site at onset; (2) a Cox regression model with survival as the outcome variable, adjusting for age at onset, sex, and site at onset; and (3) a logistic regression model with site at onset as the outcome variable, adjusting for age at onset and sex. Analyses were performed for the C282Y and H63D genotypes combined. The wild-type genotype was used as the reference value. Because 2 loci (C282Y and H63D) were studied, we considered a more conservative \( P < .025 \) as statistically significant. A Mantel-Haenszel common odds ratio (OR) estimate was computed to pool the association between HFE mutations and risk of developing ALS with the associations described in 3 previous studies.\(^{14,16}\)

Table 1 gives the characteristics of the patients and controls. The control population had a higher median age and consisted of more women. We corrected for these confounders in all our analyses.

The frequencies for both the C282Y and H63D alleles were in Hardy-Weinberg equilibrium in the control population. Table 2 summarizes and compares the genotype distributions of patients and controls. Homozygous mutations at H63D were independently associated with an increased risk of developing ALS (OR, 2.2; 95% confidence interval [CI], 1.1-4.1; \( P = .02 \)). Other genotypes were not significantly different between patients and controls. Comparing genotypes of the patient group with each control group separately provided similar results (data not shown). When our data were pooled with data from all previous HFE association studies performed in various geographical regions, an increased risk was observed for H63D mutation carriers (OR, 1.7; 95% CI, 1.1-2.5), homozygotes (OR, 2.7; 95% CI, 1.7-4.4), and heterozygotes (OR, 1.5; 95% CI, 1.0-2.1) (Figure). We also examined the extent to which a mutation in HFE influences clinical phenotype. Table 3 gives the age at onset and survival of patients with ALS together with HFE genotypes. Heterozygosity at H63D was associated with a higher age at onset (mean difference, 5.4 years; 95% CI, 2.2-8.5; \( P = .001 \)). In contrast, in the control group, H63D homozygotes, heterozygotes, and mutation carriers were similar in age to wild types (data not shown). Presence of a C282Y or H63D mutation did not affect survival (Table 3) or site at onset (data not shown).

COMMENT

In this study of 289 patients and 5886 controls, we detected a positive association between homozygosity for the H63D mutation and ALS, suggesting that HFE is a contributing factor in the development of ALS in the Dutch population. Moreover, we found heterozygosity for the H63D HFE mutation to be associated with a higher age at onset, possibly indicating that H63D is a risk factor for a later-onset form of ALS.

Our large control group was taken from prospective population-based studies\(^{19,20}\) that reflect the general Dutch population and made genotyping of a new control sample redundant. The control group differed from the patient population with regard to age and sex, but we adjusted for these confounders in our analyses. Moreover, no significant differences in HFE mutation frequencies have been reported for different age and sex groups.\(^{21,22}\) Furthermore, all patients were white, and observed genotype frequencies in the control population were similar to those reported for non-Hispanic white individuals in previous population-based studies and in Hardy-Weinberg equilibrium.\(^{1,21}\) In addition, comparison of genotypes of the patient group with each control group separately gave similar results.
Table 3. Association Between HFE Genotypes and Clinical Phenotypes of Patients With Amyotrophic Lateral Sclerosis

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mean Age at Onset, y</th>
<th>Regression Coefficient β (95% CI)</th>
<th>P Value*</th>
<th>Median Survival, y</th>
<th>HR (95% CI)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFE genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>58.2</td>
<td>1.0‡</td>
<td></td>
<td>3.2</td>
<td>1.0‡</td>
<td></td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>63.4</td>
<td>5.4 (2.2 to 8.5)</td>
<td>.001§</td>
<td>2.6</td>
<td>1.2 (0.8 to 1.7)</td>
<td>.30</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>58.4</td>
<td>−1.0 (−3.8 to 1.8)</td>
<td>.50</td>
<td>3.1</td>
<td>1.3 (0.7 to 2.4)</td>
<td>.50</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>62.4</td>
<td>1.8 (−0.4 to 3.9)</td>
<td>.10</td>
<td>3.1</td>
<td>1.0 (0.5 to 2.1)</td>
<td>.99</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>68.1</td>
<td>0.2 (−2.3 to 2.9)</td>
<td>.90</td>
<td>4.4</td>
<td>0.4 (0.1 to 2.9)</td>
<td>.40</td>
</tr>
<tr>
<td>C282Y genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>63.0</td>
<td>1.0‡</td>
<td></td>
<td>3.2</td>
<td>1.0‡</td>
<td></td>
</tr>
<tr>
<td>All C282Y carriers†</td>
<td>56.6</td>
<td>−2.8 (−7.4 to 1.9)</td>
<td>.20</td>
<td>3.1</td>
<td>1.1 (0.6 to 1.9)</td>
<td>.90</td>
</tr>
<tr>
<td>H63D genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>58.0</td>
<td>1.0‡</td>
<td></td>
<td>3.1</td>
<td>1.0‡</td>
<td></td>
</tr>
<tr>
<td>All H63D carriers†</td>
<td>62.9</td>
<td>5.2 (2.4 to 8.0)</td>
<td>&lt;.001§</td>
<td>2.6</td>
<td>1.1 (0.8 to 1.5)</td>
<td>.60</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; WT, wild type.
*The effect on age at onset was computed by linear regression adjusting for sex and site at onset of disease (first column of P values); the effect on survival was computed by Cox regression adjusting for sex at onset, age, and site at onset (second column of P values).
†Homozygotes and heterozygotes.
‡WT was used as reference value.
§P <.02.

Our findings agree with those of a previous study of 121 patients and 133 controls, which demonstrated an increased risk of developing ALS when an H63D mutation was present. This association was significant for H63D heterozygotes. A more recent study, which included 379 patients and 400 controls, showed an increased risk of developing ALS for H63D homozygotes and heterozygotes in 2 populations. In a smaller population of 51 patients and 47 controls, no difference was found in the presence of HFE mutations between ALS patients and controls. We pooled these results and showed an association for H63D homozygotes, heterozygotes, and carriers, supporting a genetic association.

Recommendations for performing genetic association studies have been published previously. By increasing the sample size, pooling data of individual studies in a meta-analysis aids in estimating population-wide effects of genetic associations. Moreover, a single significant association should be independently replicated, preferably at least twice. Therefore, the present study adds insight to conclusions from previous studies.

In our study, only H63D homozygotes demonstrated significance, whereas previous studies also showed an association with H63D heterozygotes. A difference in genetic background in the Dutch population could account for the somewhat weaker association with H63D (in heterozygotes and carriers) found in our study. Nevertheless, our meta-analysis clearly shows an association between ALS and H63D homozygotes and heterozygotes.

Several possible mechanisms could explain the observed relationship between H63D mutations and the development of ALS. Increased oxidative stress caused by excessive iron could play a role. However, the C282Y mutation, rather than H63D, is shown to have a greater effect on iron concentrations in serum and deposition in liver. An overall increase in iron supplies, therefore, is not a plausible biological mechanism in ALS. In addition, no indications were found of relevant neurological involvement in HFE-linked HH. Additional roles of HFE in other tissues still require elucidation, and H63D mutations could lead to unique conformational changes in the HFE protein that exert an effect mainly on local iron concentration at the motor neuron level. In particular, it has been proposed that H63D mutations predominantly affect the binding of HFE to the transferrin receptor, which plays a role in neuronal iron uptake. Studies in patients with Alzheimer disease support a role for the transferrin receptor in neurodegeneration. Alternatively, H63D is in linkage disequilibrium with other genetic variants that may initiate pathological cellular processes.

In conclusion, the findings suggest a role for HFE mutations in the development of ALS, although caution should be used in estimating the size of the effect. Further independent HFE genotype association studies are needed in different geographical regions. Moreover, serum iron values could provide further clues about the possible role for disorders of iron metabolism in patients with ALS.

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REFERENCES


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