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Negative results

H63D polymorphism in *HFE* is not associated with amyotrophic lateral sclerosis

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ABSTRACT

The H63D polymorphism in *HFE* has frequently been associated with susceptibility to amyotrophic lateral sclerosis (ALS). Regarding the role of *HFE* in iron homeostasis, iron accumulation is considered an important process in ALS. Furthermore, novel therapeutic strategies are being developed targeting this process. Evidence for this genetic association is, however, limited to several small studies. For this reason we studied the H63D polymorphism in a large European cohort including 3962 ALS patients and 5072 control subjects from 7 countries. After meta-analysis of previous studies and current findings we conclude that the H63D polymorphism in *HFE* is not associated with susceptibility to ALS, age at disease onset, or survival.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting motor neurons in the brain and spinal cord. Patients suffer from progressive paralysis and generally succumb within 3 to 5 years after disease onset because of respiratory insufficiency (Hardiman et al., 2011). The etiology of ALS

remains unknown and disease-modifying treatments are limited. A publicly available online meta-analysis of all genes studied in ALS (www.alsgene.org) identified the H63D polymorphism in *HFE* as one of the strongest susceptibility factors in ALS (Lill et al., 2010). Considering the role of *HFE* in iron homeostasis, iron accumulation is considered a possible mechanism contributing to ALS pathophysiology (Kwan et al., 2012; Rothstein, 2009). Moreover, this process has become a target for novel therapeutic strategies (Kupersmidt et al., 2011; Weinreb et al., 2011).

The studies that form the genetic basis for these strategies consistently report an increased risk of ALS for carriers of the H63D substitution (Goodall et al., 2005; He et al., 2011; Restagno et al., 2007; Sutedja et al., 2007; Wang et al., 2004). The individual studies

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Fig. 1. (A) Meta-analysis of previous studies. (B) Meta-analysis of results in previous and current studies. Abbreviations: Bel, Belgium; Chin, China; CI, confidence interval; FR, France; Ger, Germany; IR, Ireland; It, Italy; NL, the Netherlands; OR, odds ratio; UK, United Kingdom; US, United States.

are, however, moderately powered and as a result display wide confidence intervals for their effect size. Moreover, this association could not be replicated in a recent French study (Fig. 1A) (Praline et al., 2012).

For this reason we have conducted a genetic association study in a large European cohort including 7 different populations to definitely determine whether the H63D polymorphism in *HFE* is indeed associated with ALS.

2. Methods

Patients were recruited from outpatient clinics specializing in neuromuscular diseases at the participating centers from 7 different countries (the Netherlands, Belgium, Germany, United Kingdom, Ireland, Sweden, and Switzerland). Control subjects were unrelated individuals, free of any neuromuscular disease. We genotyped patients and control subjects using a predesigned TaqMan assay (Applied Biosystems, Foster City, CA, USA). The association between the H63D polymorphism and ALS was evaluated by logistic regression, correcting for country. An effect on age at onset was evaluated using linear regression, correcting for site of onset (spinal vs. bulbar) and sex. We tested the association with survival in a Cox proportional hazards model correcting for age at onset, site of onset, and sex.

3. Results

In total we included 3962 ALS patients and 5072 healthy control subjects (Supplementary Table 1). There was no difference in the H63D allele frequency between patients and control subjects (Supplementary Table 2; odds ratio, 1.00; 95% confidence interval, 0.92–1.09). Combining our data with those of previous studies (5894 patients and 12,379 control subjects) again did not result in a significant association (Fig. 1B; odds ratio, 1.06; 95% confidence interval, 0.98–1.14). We did not observe an effect of the H63D polymorphism on age at onset ($p = 0.62$ and $p = 0.68$ for wild-type/H63D and H63D/H63D genotypes respectively). Additionally, there was no effect on survival ($p = 0.26$ and $p = 0.51$ for wild-type/H63D and H63D/H63D genotypes respectively; Supplementary Fig. 2).

4. Discussion

In a well-powered (99.98% to detect the previously described effect) cohort we did not observe the association between the H63D polymorphism and ALS.

With the exception of a recent French study, these results contrast sharply with earlier studies on *HFE* in ALS, reporting a considerable effect size (Goodall et al., 2005; He et al., 2011; Praline et al., 2012; Restagno et al., 2007; Sutedja et al., 2007; Wang et al., 2004). Because our study population was derived from 7 different populations, including those from Ireland and the United Kingdom in which an association was previously found, a population-specific association cannot explain our findings (Goodall et al., 2005). Most likely, previous studies have suffered from publication bias favoring representation of a positive association. This bias is even more pronounced in smaller studies. A coincidental positive association in these studies has a higher chance of publication, and studies that fail to find an association will be criticized for lack of statistical power. This kind of bias highlights the importance of well-powered genetic association studies regardless of the association found.

In view of our findings, the perceived role of altered iron metabolism in ALS might need to be revised. Although there are data from imaging studies that suggest iron accumulation in ALS, and biochemical studies that indicate the presence of abnormal iron ligands in cerebrospinal fluid, the mechanisms of these changes remain to be elucidated, and it is possible that they are secondary rather than primary events in ALS (Ignjatovic et al., 2012; Kwan et al., 2012).

In conclusion, our data show that the H63D polymorphism in *HFE* is not a risk factor for ALS. The role of *HFE* in ALS pathogenesis, therefore, needs to be reconsidered. Furthermore, our data highlights the importance of large-scale genetic association studies in ALS.

Disclosure statement

The authors report no conflict of interest.

All participants gave written informed consent and the Medical Ethics Review Boards of the participating institutions approved this study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2012.07.020>.