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HFE H63D polymorphism is increased in patients with amyotrophic lateral sclerosis of Italian origin

A role for metal-mediated oxidative stress in the pathogenesis of amyotrophic lateral sclerosis (ALS) was proposed in 1994 in the first studies of familial ALS mutant superoxide dismutase 1, and interference with iron homoeostasis is now postulated.[1] The HFE gene on chromosome 6 is a mean corpuscular haemoglobin class I-like molecule related to iron regulation. Mutations in the coding region cause hereditary haemochromatosis, a common autosomal recessive disorder of iron metabolism that leads to iron overload in adulthood. Recent reports on HFE mutations in ALS showed contradictory results. Two studies described a higher prevalence of the HFE mutations in ALS than in the control group, and one study did not find any difference between the patients with ALS and the control group.[2–4] We analysed a series of Italian patients with ALS to investigate whether mutations in the HFE gene could represent a risk factor for ALS.

A total of 149 sporadic Italian patients with ALS (mean (standard deviation (SD)) age 59.4 (9.7) years) according to El Escorial criteria for clinically definite or probable ALS were consecutively recruited to this study. Control samples were obtained from 168 healthy people, matched by age (difference of 5 years), sex and ethnic origin (Italian region of birth) to patients with ALS. Patients and controls were informed about the objectives of the study, and informed consent was obtained. The study was approved by the institutional ethics committee. Blood samples were collected, and DNA was purified with a 6100 Nucleic acid Prep Station (ABI PRISM™). Rapid detection of H63D, C282Y and S65C, the three most common mutations in the HFE gene, was performed using the pyrosequencing technique (Pyrosequencing AB, Uppsala, Sweden). This assay is based on a duplex polymerase chain reaction (PCR) in which exons 2 and 4 are amplified together. The exon 2 PCR product is used for S65C and H63D polymorphisms, and the exon 4 PCR product for the C282Y mutation. The mutation analysis was subsequently carried out in a triplex assay by means of three pyrosequencing primers in one well. Forward PCR primers for each reaction were modified with biotin at the 5′ terminus to capture single-stranded templates from PCR products for pyrosequencing. The following PCR primers were used: for exon 2, ex2-F 5′-ggc tac gtg gat gac cag c-3′ and ex2-R 5′-gag ttc ggg gct cca cac-3′; for exon 4 ex4-F 5′-cct ggg gaa gag cag aga t-3′ and ex4-R 5′-cag atc aca atg agg ggc tg-3′. The primers used for sequencing were: 5′-gct gtt cgt gtt cta tg-3′ for exon 2 and 5′-ggg gaa gag cag aga t-3′ for exon 4. The PCRs were performed for 45 cycles, with initial denaturation at 95℃ for 10 min, followed by 95℃ for 30 s, annealing for 30 s and extension at 72℃ for 30 s. The final extension was at 72℃ for 5 min. Bound biotinylated single-stranded DNA was prepared following the protocols provided by the manufacturer (PSQ96 sample preparation kit; Pyrosequencing AB). SNP/AQ analysis was performed automatically on a PSQ™96 MA system using enzymes and reagents from an SNP Reagent kit (Pyrosequencing AB).

The group with ALS included 65 women and 84 men (mean (SD) age 61.1 (11.1) years), and the control group included 66 women and 102 men (mean (SD) age 60.7 (9.2) years). Table 1 shows the findings for the three SNPs H63D, C282Y and S65C. Analysis of HFE mutations showed a higher frequency of the mutated allele H63D in patients with ALS than in controls (28.8% *v* 14.8%; $p = 0.004$). The odds ratio conferred by the presence of the H63D allele was 2.25 (95% confidence interval 1.30 to 3.93). An increased frequency was also found in patients with ALS when all three mutations were combined (33.3% *v* 17.3%; $p = 0.002$). No significant differences were found between patients with the H63D allele and patients with wild-type HFE gene considering age of onset (63.4 (SD 9.3) *v* 60.2 (SD 11.9)), sex (22 men and 21 women *v* 62 men and 44 women), type of onset (33 spinal and 10 bulbar *v* 80 spinal and 26 bulbar) and disease duration (median survival time, 783 *v* 993 days).

Our data support the hypothesis that the change in iron metabolism may confer susceptibility to neurodegenerative diseases such as ALS. Our results are consistent with those found in the USA,[2] and in Ireland and Britain.[4] Interestingly, the second study reported an odds ratio of 1.85 (95% confidence interval 1.35 to 2.54) for the presence of the heterozygous H63D polymorphism, a value similar to that found in our population. In Europe, the C282Y allele has a north to west frequency-decreasing gradient, with higher frequencies reported in Ireland (28.4%) and Britain (17.4%) and lower frequencies in Italy (3.2%) and Greece (2.6%). Conversely, the H63D allele has a higher frequency in southern Europe (Spain, 32.3%) and a lower frequency in the Celtic populations (5%).[5] These marked ethnic differences may explain the negative findings of one study on patients with ALS in Texas, USA,[3] in which no matching for ethnic origin was performed.

The possible role of the H63D polymorphism in ALS is unclear. In a human neuronal cell line transfected with genes carrying the HFE mutations, the H63D polymorphism induced a decreased expression of SOD1, α-tubulin and β-actin;[2] these events can cause a disruption of axonal transport, a factor implicated in ALS pathogenesis. Alternatively, the H63D polymorphism may determine a subclinical increase in intracellular iron, possibly related to neurone oxidative damage. Further studies on the analysis of iron metabolism in patients with ALS are needed to elucidate the role of the H63D polymorphism as a genetic risk factor for sporadic ALS. An alternative possibility could be a linkage disequilibrium of ALS with an unknown gene located near the HFE locus.

Table 1 Frequency of HFE polymorphisms in patients with amyotrophic lateral sclerosis and controls

	Patients with ALS		Controls	
Polymorphisms	**No**	**%**	**No**	**%**
H63D/wild type	41	27.5	25	14.8
H63D/H63D	2	1.3	–	–
C282Y/wild type	5	3.3	3	1.8
S65C/wild type	2	1.3	1	0.6
Wild/wild type	99	66.4	139	82.7
Total	149	100	168	100

AlS, amyotrophic lateral sclerosis.

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Causes of death in multiple system atrophy

Multiple system atrophy (MSA) is a heterogeneous neurodegenerative disorder, with a clinical presentation combining extrapyramidal,