The HFE p.His63Asp polymorphism modifies ALS outcome in patients with SOD1 mutations

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Background

Recently, the His67Asp polymorphism of the HFE gene (analogue of the His63Asp human polymorphism) has been observed to negatively influence the survival of the SOD1 transgenic mouse (Nandar et al., 2014). In a previous study we have found that this polymorphism did not influence ALS phenotype and survival in a large series of Italian ALS patients, with the possible exception of patients carrying SOD1 mutations (Chiò et al., 2015). However, the number of SOD1 patients in that series was relatively small (n=26).

Aim

To assess whether the HFE rs1799945 (p.His63Asp) common polymorphism is a modifier of phenotype and survival in a large series of Italian and French SOD1-mutated patients.

Methods

The exon2 of HFE was amplified by PCR and analyzed by Denaturing High-Performance Liquid Chromatography (DHPLC) (Transgenomic, Inc., Omaha, NE, USA). PCR products with heteroduplex profiles were sequenced on an ABI 3500 sequencer (Life Technologies, Foster City, CA, USA) with BigDye termination v.1.1 (Life Technologies) technologies according to standard protocol. Samples with homozygous profiles were coupled with a wild-type reference, denaturated and re-analyzed by DHPLC in order to detect also homozygous sequence alterations. If a mixed profile was positive, the original sample was sequenced. All sequencing products were analysed with SeqScape Software v.3.0 (Applied Biosystems - Life Technologies).

Results

A total of 185 Italian and French patients carrying different mutations of the SOD1 gene were assessed for the rs1799945 polymorphism of the HFE gene. The following allelic frequencies were found: CC 127 cases (68.6%), GC 53 cases (28.6%), and GG 5 cases (2.7%). The p.His63Asp polymorphism did not influence age at onset (CG + GG, 53.0 years, SD 11.33; CC, 54.0 SD 12.4; p=0.62) and site of onset. In univariate analysis, patients carrying the H63D (CG + GG) polymorphism had a longer median tracheostomy-free survival (median survival, 9.8 years, 95% c.i. 8.1-11.5, vs. 7.3 years, 95% c.i. 6.3-8.4; p=0.031).

The presence of the p.His63Asp polymorphism remained significant also in Cox multivariable analysis using as covariates age at onset, site of onset, positive family history, nation and severity of mutations (hazard ratio, 0.52, 95% CI 0.32-0.85, p=0.01).

Discussion

In patients with SOD1 mutations the presence of a G allele of the HFE gene was found to be significantly associated with a longer survival. This finding is in contrast with the reported shorter survival in the double transgenic mouse line (SOD1/His67Asp) (Nandar et al., 2014), highlighting the possibility that genetic interactions in mice compared with humans are biologically different. The mechanism through which the His63Asp HFE polymorphism modifies the outcome of SOD1 ALS remains to be elucidated.

References

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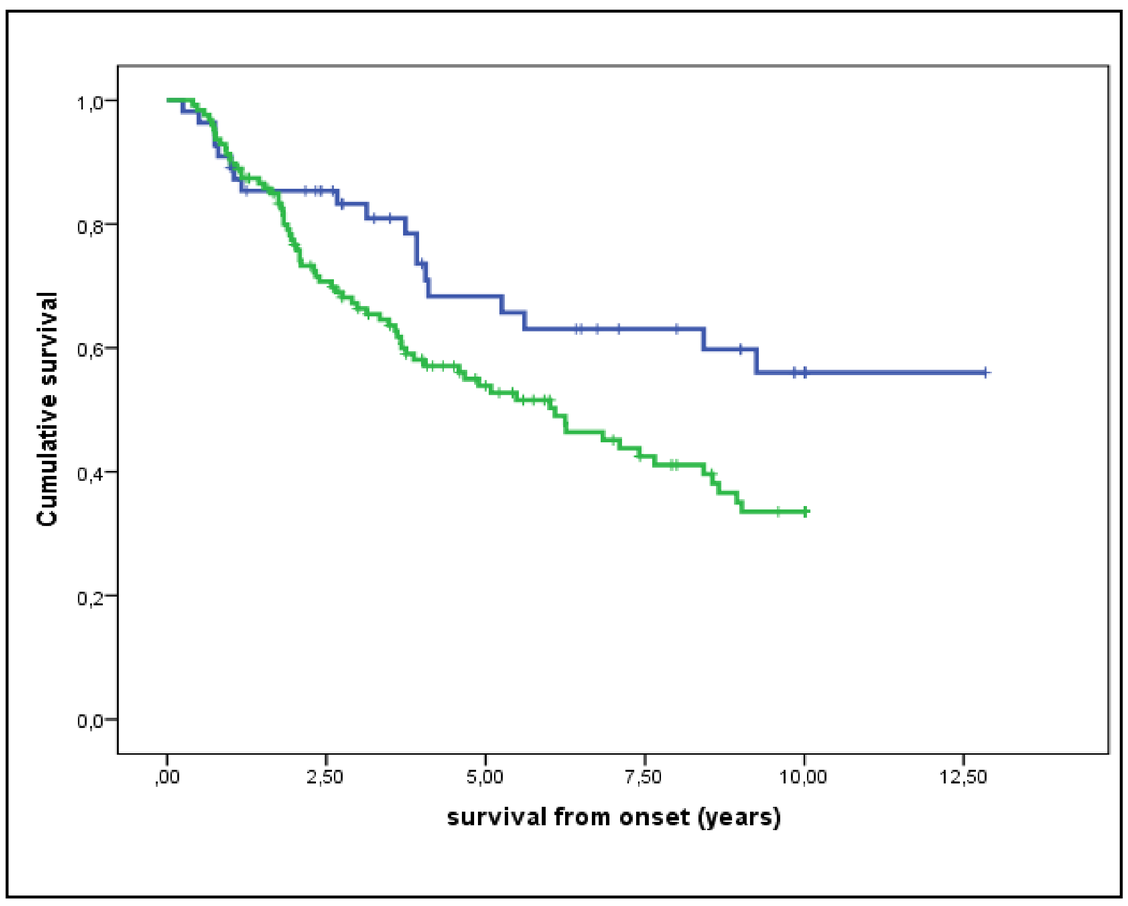

Mutation	Frequency	Percentage	Mutation	Frequency	Percentage
G93D	25	13,5%	N19S	5	2,7%
D90Ahete	14	7,6%	G93C	4	2,2%
L144F	14	7,6%	S134N	4	2,2%
L84F	11	5,9%	T137A	3	1,6%
G41S	10	5,4%	A95G	2	1,1%
A4V	9	4,9%	D109Y	2	1,1%
D11Y	6	3,2%	D124G	2	1,1%
E133del	6	3,2%	G10R	2	1,1%
N65S	6	3,2%	G147D	2	1,1%
D90Ahomo	5	2,7%	G147S	2	1,1%
G72S	5	2,7%	I113T	2	1,1%
I149T	5	2,7%	Other	39	21,0%

Genotypes	Observed #	Expected #
CC	127	127,4
CG	53	52,3
GG	5	5,4
Minor allele frequency	0,17	
C^2 =	0,035786435	
C^2 test *P* value =	0,849957 with 1 degree of freedom.	

Figure 1. Cumulative survival of patients carrying the H63D (CG + GG) polymorphism [blue line] vs. wild type patients [green line]. **Table 1.** SOD-1 mutation list in order of frequency in our series. **Table 2.** Calculation of Hardy-Weimberg equilibrium for different genotypes.