ALS phenotype according to HFE p.His63Asp polymorphism: an Italian multicentre study

Cammarosano S[1], Calvo A[1,2], Moglia C[1], Fuda G[1], Sabatelli M [4], Zollino M[5], ITALSGEN, Penco S [6], Lunetta C [7], Mora G [8], Battistini S [9], Mandrioli J [10], Restagno G[11], Brunetti M[11], Barberis M [11], Chiò A[1,2,3]

[1]Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy; [2]Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Turin, Italy; [3]Neuroscience Institute of Torino (NIT), Turin, Italy; [4]Neurological Institute Catholic University and I.CO.M.M. Association for ALS Research, Rome, Italy; [5]Molecular Genetics Laboratory, Department of Laboratory Medicine, Catholic University, Rome, Italy; [6]Department of Laboratory Medicine, Medical Genetics, Niguarda Ca'Granda Hospital, Milan, Italy; [7]Neuromuscolar Omnicentre, Fondazione Serena ONLUS, Niguarda Ca'Granda Hospital, Milan, Italy; [8]Salvatore Maugeri Foundation, IRCSS, Scientific Institute of Milano, Milano Italy; [9]Department of Neuroscience, Section of Neurology, University of Siena, Siena, Italy; [10]Department of Neuroscience, Sant'Agostino Estense Hospital, University of Modena, Modena, Italy; [11]Molecular Genetics Unit, Department of Clinical Pathology, Azienda Ospedaliera Ospedale Infantile Regina Margherita Sant Anna, Turin, Italy.

Objective

To assess the influence of the p.H63D polymorphism of the HFE gene on the phenotype and survival of a large series of ALS patients of Italian and Sardinian ancestry.

Background

Polymorphisms of Unc-13 homolog A (UNC13A) (Diekstra et al, 2012; Chiò et al, 2013), of Non-Imprinted in Prader–Willi/Angelman syndrome 1 (NI-PA1) (Blauw et al, 2014) genes and of polyQ intermediate-length expansion of ATXN2 (Chiò et al, 2014) have been associated with a shorter survival, while a locus on 1p34.128 has been associated to a younger age at onset (ALSGEN 2013).
Recently, it has been reported that the p.His63Asp polymorphism of the HFE gene accelerates disease progression in the ALS SOD1 transgenic mouse (Nandar et al, 2014). Conversely, in a small study on 35 ALS patients, it has been reported that patients carrying the p.H63D polymorphism of the HFE gene had a significantly longer survival than those with the wild type gene (Su et al, 2013).

Methods

ALS cases were collected through the Italian ALS Genetic (ITALSGEN) and the Sardinian ALS Genetic (SARDINIALS) consortia (Chiò et al, 2012; Chiò et al, 2014). Cases are patients with definite, probable, probable-laboratory supported and possible ALS diagnosed between 2006 and 2012. A total of 149 cases have been already reported (Restagno et al, 2007). All cases were also screened for most common ALS genes, i.e. C9ORF72, SOD1, TARDBP and FUS. Controls were 1302 Italian and 121 Sardinian subjects without neurological disorders, age- and gender-matched to cases. Of these, 162 Italian subjects have been previously reported (Restagno et al, 2007).
Samples were genotyped using the Illumina NeuroX SNP array. Statistical comparisons between means were made with Student's t-test or analysis of variance (ANOVA); comparison between categorical variables was made with $\chi 2$ test; Levene's test was used to confirm the equality of variances. Survival was calculated from onset to death/tracheostomy or censoring date (October 31, 2014), using the Kaplan-Meier survival modelling, and differences in survival were measured by the log-rank test. No patients were lost to follow-up. Multivariable analysis was performed with the Cox proportional hazards model (stepwise backward) with a retention criterion of $p<0.1$. Significance level was set at $p<0.05$. All patients and controls signed a written informed consent.

Genotype	CC	GC	GG	Total
Italians				
Cases	804 (71.8%)	293 (26.2%)	22 (2.0%)	1119
Controls	948 (72.8%)	322 (24.7%)	32 (2.5%)	1302
Sardinians				
Cases	154 (66.4%)	70 (30.2%)	8 (3.4%)	232
Controls	79 (65.3%)	38 (31.4%)	4 (3.3%)	121

Table 1. Frequency of HFE genotypes in Italian and Sardinian ALS patients and controls

	CC	GC	GG	P value
Mean age at onset	**Italians**			
	62.3 (11.2)	62.2 (11.7)	62.5 (11.2)	0.92
	Sardinians			
	60.2 (12.8)	60.6 (10.5)	65.4 (10.3)	0.78

Table 2. Mean age at onset according to HFE H63D genotype.

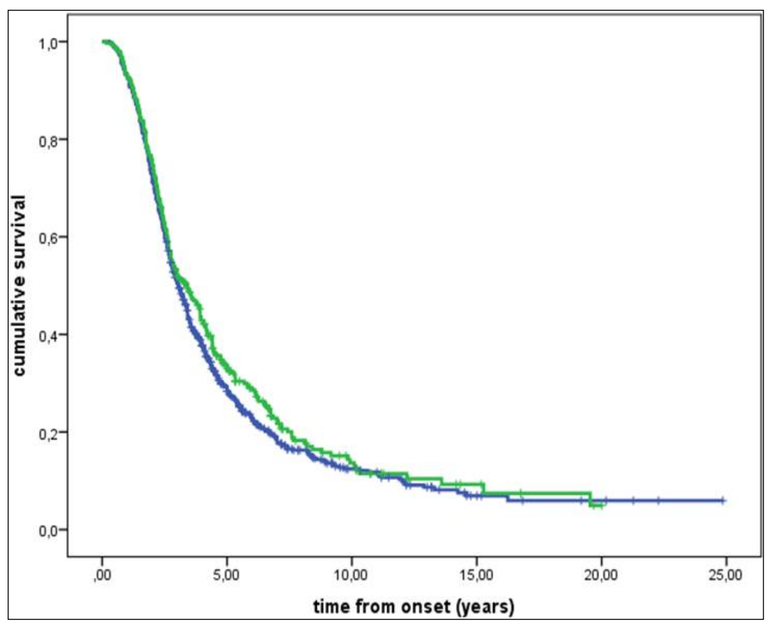

Figure 1. Italian patients. Survival curves by HFE genotype. CC, median survival time 3.0 years (interquartile range 1.9-5.5); GC/GG, median survival time 3.4 years (interquartile range 2.0-6.7). p=n.s. CC, blue; GC/GG green

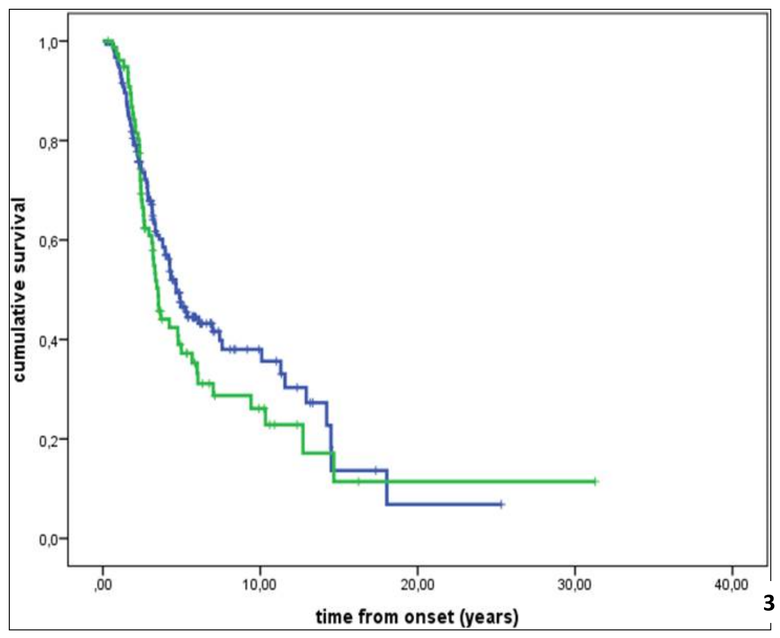

Figure 2. Sardinian patients. Survival curves by HFE genotype. CC, median survival time 4.7 years (interquartile range 2.4-14.2); GC/GG, median survival time 3.5 years (interquartile range 2.3-10.3). p=n.s. CC, blue; GC/GG green.

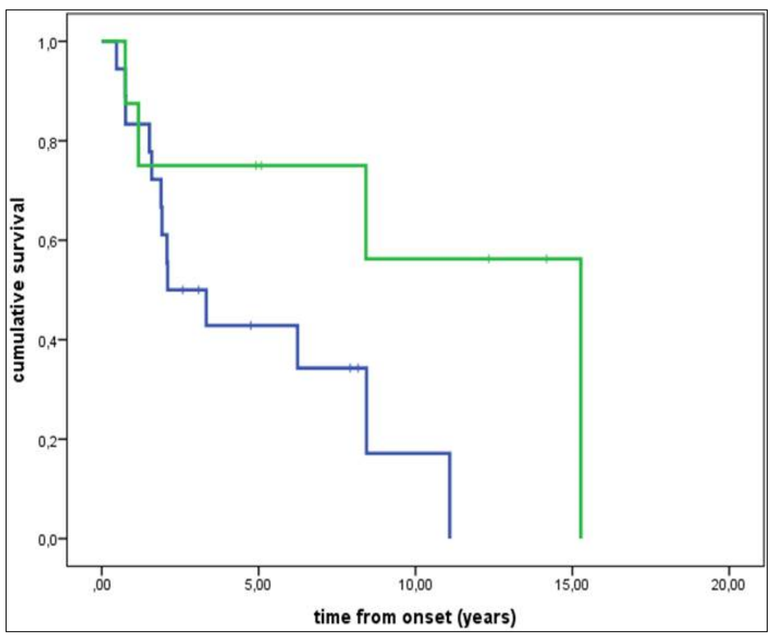

Figure 3. Italian patients carrying SOD1 mutations. CC, median survival time 2.1 years (interquartile range 2.6-8.4); GC/GG, median survival time 15.3 years (interquartile range 1.2-15.3). p=0.04. CC, blue; GC/GG green

Results

A total of 1119 Italian and 232 Sardinian ALS patients have been included in the study. Patients' clinical characteristics and genetic mutations are reported in Table 1. The frequency of CC, GC and GG genotypes in Italian and Sardinian ALS cases and controls is reported in Table 1. No significant differences were found in either populations. Patients with CC, GC and GG genotypes did not differ by age at onset and site of onset (Tables 2 and 3). No difference of survival was found both considering the CC/GC/GG phenotypes and the presence of a G allele in either cohorts of patients (Figures 1 and 2). This finding has been confirmed in Cox multivariable analyses.
We also assessed the possible effect of HFE phenotypes in patients carrying genetic mutations. A list of identified genetic mutations is reported in the Supplementary Table. No difference was found in the groups of patients carrying C9ORF72, FUS and TARDBP mutations. In the 26 patients with SOD1 mutations we found an increased survival in patients with GC or GG compared to CC genotypes or in patients carrying the G allele (dominant assumption) ($p=0.04$) (Figure 3). This finding is confirmed by the multivariable Cox model, where the G is retained as an independent prognostic factor ($p=0.03$).

Conclusions

In two cohorts of Italian and Sardinian patients we have found that the p.H63D polymorphism of the HFE gene does not represent a risk factor for ALS. Moreover, we showed that the presence of the G allele does not modify overall patients' clinical phenotype and survival. However, patients with SOD1 mutations carrying the G allele had a better survival than other patients. In subjects with C9ORF72, TARDBP and FUS mutations the p.H63D polymorphism did not modify phenotype and survival.
In our series we found that in both populations the presence of a G allele or GG/GC phenotypes did not influence overall patients survival. We also looked at the patients carrying mutations of major ALS genes. No effect of HFE status was found in patients with C9ORF72, TARDBP and FUS mutations. Conversely, in patients with SOD1 mutations the presence of a G allele was significantly associated with a longer survival. This finding is in contrast with the reported shorter survival in the double transgenic mouse line (SOD1/H67D) (Nandar et al, 2014), highlighting the possibility that genetic interactions in mice compared to humans are biologically different. Although based on a small cohort of patients, this interaction warrants further studies to better understand the genetic mechanisms underlying ALS.

Site of onset	CC	GC	GG	P value
Italians				
Bulbar	216	78	5	0.91
Spinal	588	215	17	
Sardinians				
Bulbar	31	15	2	0.93
Spinal	123	55	6	

Table 3. Site of symptom onset according to HFE H63D genotype

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