# CHCH10 mutations in an Italian cohort of familial and sporadic amyotrophic lateral sclerosis patients

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# *CHCH10* mutations in an Italian cohort of familial and sporadic ALS patients

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## Abstract

Mutations in *CHCHD10* have recently been described as a cause of frontotemporal dementia (FTD) co-morbid with amyotrophic lateral sclerosis (ALS). The aim of this study was to assess the frequency and clinical characteristics of *CHCHD10* mutations in Italian patients diagnosed with familial (n = 64) and apparently sporadic ALS (n = 224). Three apparently sporadic patients were found to carry c.100C>T (p.Pro34Ser) heterozygous variant in the exon 2 of *CHCHD10*. This mutation had been previously described in two unrelated French patients with FTD-ALS. However, our patients had a typical ALS, without evidence of FTD, cerebellar or extrapyramidal

signs, or sensorineural deficits. We confirm that *CHCHD10* mutations account for ∼1% of Italian ALS patients and are a cause of disease in subject without dementia or other atypical clinical signs.

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder affecting motor neurons and clinically characterized by paralysis and respiratory failure leading to death, typically within 3 to 5 years of symptom onset. Approximately 10% of patients have a family history for ALS or frontotemporal dementia (FTD). The genetic etiology of two thirds of these cases has been identified, with mutations in *SOD1, TARDBP* and *FUS*, as well as the pathogenic repeat expansion in *C9ORF72*, being the most common causes (Chiò et al, 2013; Renton et al, 2014).

Recently, a missense mutation in the *coiled-coil-helix-coiled-coil-helix domain containing 10* (*CHCHD10*) gene on chromosome 22q.11.23 was reported to cause FTD-ALS in a large French pedigree (Banwarth et al, 2014). Additional mutations were subsequently reported in ALS pedigrees without cognitive impairment (Müller et al, 2014; Johnson et al, 2014). However, the importance of *CHCHD10* mutations as a cause of ALS remains unclear. The aim of the current study is to determine the frequency of *CHCHD10* mutations in a cohort of familial (fALS) and sporadic (sALS) Italian ALS patients.

## 2. Methods

### 2.1 Samples

Samples includes (a) 64 unrelated Italian probands with familial ALS (fALS) recruited through the Italian ALS Genetic (ITALSGEN) consortium; (b) 224 apparently sporadic Italian ALS cases (sALS) diagnosed between June 2012 and June 2014 and residing in Piemonte. These cases were identified through the Piemonte and Valle d'Aosta registry for ALS (PARALS) (Chio et al, 2012); and (c) 165 healthy Italian controls that were age- and gender-matched to patients. These individuals were recruited using the list of the patients attending the same general practitioners as the sporadic ALS patients. ALS cases were negative for mutations in *SOD1, TARDBP* and *FUS*, and did not carry the *C9ORF72* pathogenic repeat expansion.

Patients with definite, probable, probable-laboratory supported or possible ALS were included in the analysis (Brooks et al, 2000). All cases were tested for cognitive impairment using an extensive test battery (listed in Appendix) (Strong et al, 2009; Montuschi et al, 2014).

### 2.2 Sequencing of CHCHD10

Coding exons and flanking intronic regions of *CHCHD10* (NM_213720.2) were amplified by PCR and analyzed by DHPLC (Transgenomic, Inc., Omaha, NE, USA). PCR products with abnormal heteroduplex profiles were sequenced on an ABI 3130 sequencer (Life Technologies, Foster City, CA, USA). Primer sequences and PCR conditions are listed in the Appendix.

### 2.3 Standard Protocol Approvals and Patient Consents

The ethical committees of the recruiting centers approved the study. All patients and control subjects proved written informed consent. Databases were treated according to the Italian regulations for privacy.

## 3. Results

Demographic and clinical characteristics of the ALS patients and controls are reported in Table 1. In our screening of the 288 ALS patients, we found seven cases carrying four distinct variants in *CHCHD10* (Table 2). Of these, a c.100C>T heterozygous variant in the exon 2 leading to the substitution of a serine for a proline residue (p.Pro34Ser) was found in three apparently sporadic ALS cases. This mutation was not present in online databases of human polymorphisms including dbSNP (build 138), the 1000 Genomes database (phase 3 release), and the 60,706 cases of the Exome Aggregation Consortium (ExAC, exac.broadinstitute.org). *In silico* analysis (polyphen) predicted that this amino acid change was damaging to protein function.

Other genetic variants identified in *CHCHD10* in our Italian cohort were: c.234G>A (p.Ser78Ser), c.274G>A (p.Ala92Thr), c.286C>A (p.Pro96Thr), and c.312C>T (P.Tyr104Tyr). There variants were of unclear pathogenicity as they were also present in Italian controls, online databases of human polymorphisms, or were predicted to result in benign changes by *in silico* analysis.

### 3.1 Clinical description of patients carrying p.Pro34Ser CHCHD10 mutation

The first patient was a 69-year-old woman who presented with dysarthria and dysphagia. Neurological examination performed six months after symptom onset found tongue atrophy with a positive jaw jerk, atrophy and weakness of the small muscles of the hand, and generalized hyperreflexia. Neurophysiological examination showed diffuse signs of active and chronic denervation. Neuropsychological testing was normal. Familial history was negative for ALS or FTD: her father died at age 57 from lung cancer and her mother at 61 due to breast cancer. Her two siblings were negative for neurological disorders. She died from respiratory failure 18 months after symptom onset.

The second patient developed weakness of his right shoulder at 58 years of age. Neurological examination revealed marked atrophy and weakness of both shoulder girdles (more marked on the right side). Deep tendon reflexes were normal in the upper limbs and hyperreflexic in lower limbs. Babinski and Hoffman signs were not present. Cervical MRI was normal and neurophysiological examination demonstrated chronic denervation of cervical region. He was cognitively normal. Family history was negative for ALS. However, his 94 years old mother was alive and affected by progressive gait impairment of unclear etiology.

The third patient was a 44-year-old woman who presented with dysarthria. Neurological examinations performed three months after symptom onset revealed, tongue atrophy and fasciculations, weakness and hypotrophy of small hand muscles, and generalized hyperreflexia. Neurophysiological testing showed diffuse signs of active and chronic

denervation. Neuropsychological examination was normal. Familial history was negative for ALS or FTD: her father died at 72 years of age due to cirrhosis and her mother died at 56 due to cerebral hemorrhage. Her six siblings were negative for neurological disorders. She died from respiratory failure fifteen months after symptom onset.

## 4. Discussion

We have found that ∼1% of our Italian series of ALS patients carried the p.Pro34Ser mutation of *CHCHD10*. This mutation has been already described in two unrelated French patients with FTD-ALS (Chaussenot et al, 2014). However, in contrast to the previously reported patients, our cases manifested classic ALS, and all three patients were cognitively normal without cerebellar, extrapyramidal signs or sensorineural deficits. Nevertheless, the clinical picture of our patients was heterogeneous: two patients manifested rapid clinical deterioration whereas the third case had a relatively mild course; two of patients had a predominantly bulbar phenotype, similar to the original pedigree (Bannwarth et al, 2014), whereas the third patient presented with limb-onset disease.

Our findings are consistent with previous reports. The first patients carrying mutations of this gene had a pure FTD or FTD-ALS phenotype (Bannwarth et al, 2014; Chaussenot et al, 2014). More recently, two missense mutations in the *CHCHD10* gene have been reported in patients with pure ALS, confirming that mutations of this gene can be associated with typical familial ALS without cognitive involvement and account for 1 to 2% of fALS.

To date, pathogenetic mutations of the *CHCHD10* gene are concentrated in exon 2, which encodes the non-structured N-terminal region and a highly hydrophobic helix (Gly43 to Ala 68) that may act as an interface with another protein (Chaussenot et al, 2014). *CHCHD10* protein activity is related to mitochondrial function, most notably the maintenance of mitochondrial integrity, and this may represent an interesting target for future therapeutic development.

Our data provide strong support for the pathogenicity of *CHCHD10* in ALS, broadens the phenotype associated with mutations in this gene, and suggests that certain mutations are associated with reduced penetrance. The relatively high frequency of *CHCHD10* mutations in our series indicated that it should be screened both in fALS and in apparently sALS patients.

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# Appendix. Primer sequences and PCR conditions

## Primers

| | |
|---|---|
| CHCHD10 Exon 1Fnew | GGAGAAGGGGGATAGGGTTG |
| CHCHD10 Exon 1Rnew | agcagcagccaaggtcactc |
| CHCHD10 Exon 2F | CTCCTCACTGGACACTTGGG |
| CHCHD10 Exon 2R | GGTCGTTTCCAGGAGCTG |
| CHCHD10 Exon 3Fnew | aggtggccccaggtttgaa |
| CHCHD10 Exon 3Rnew | aggtgcaagaggagggttgg |
| CHCHD10 Exon 4F | ACCTCATCAGCCAGGGAG |
| CHCHD10 Exon 4R | CCAACCCTCCTCTTGCAC |

## PCR conditions

PCR MIX Exons 1-3-4 (1ul DNA, 10 ng/ul)

| | | |
|---|---|---|
| 2× | Roche Master Mix | 12.5 ul |
| 50μM | Forward Primer | 0.2 ul |
| 50μM | Reverse Primer | 0.2 ul |
| N/A | $H_2O$ | 11.1 ul |

PCR MIX for exon2 (1ul DNA, 10 ng/ul)

| | | |
|---|---|---|
| 2× | Roche Master Mix | 12.5 ul |
| 50μM | Forward Primer | 0.2 ul |
| 50μM | Reverse Primer | 0.2 ul |
| Betaine (5M) | | 5 ul |
| N/A | $H_2O$ | 6.1 ul |

Amplification of exons 1,3,4 was performed using Roche Master mix in a touch-down PCR protocol with an initial denaturation at 94°C for 4 min, followed by 25 cycles of denaturation at 94°C for 30 sec, annealing at 65°C In the first cycle with 1.0°C decremental in each subsequent cycle for 30 sec, and elongation at 72°C for 45 sec. This was followed by 20 cycles at 94°C for 30sec, 55°C for 30 sec, and 72°C for 45 sec, with a final step at 72°C for 15 min. To enhance the formation of heteroduplex for DHPLC analysis, samples were denaturated at 95°C for 1 min, and then slowly cooled for 30 cycles at rate of 1°C/cycle.

For the exon2 the touch-down protocol was modified using 20 cycles with annealing at 66°C and decremental of 0.5°C/cycle followed by 25 cycles at annealing at 56°C; chemical conditions for amplification were adjusted by adding betaine in the standard protocol, as listed in table below.

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## Highlights

This is the first paper reporting the new discovered gene CHCHD10 in a large series of Italian familial and sporadic ALS patients. It demonstrates the mutation of this gene can be detected in apparently sporadic ALS patients with a ‘typical’ clinical picture, i.e. without dementia or cerebellar signs.

## Table 1
**Demographic and clinical characteristics of cases and controls**

| | fALS n=64 | sALS n=224 | Controls n=165 |
|---|---|---|---|
| Age at onset | 58.3 (10.4) | 65.9 (11.4) | 65.1 (10.1) |
| Gender (women, %) | 18 (28.1%) | 101 (45.1%) | 73 (44.2%) |
| Site of onset (bulbar, %) | 21 (32.8%) | 68 (30.3%) | - |
| FTD | 10 (15.7%) | 31 (14.8%) | - |

sALS, sporadic ALS; fALS, familial ALS; FTD, frontotemporal dementia

**Table 2**

**Genetic variants observed only in cases**

| case | variation | Age at onset | Gender | Site of onset | fALS/sALS | UMN/LMN | Disease duration | Exon | Effect |
|---|---|---|---|---|---|---|---|---|---|
| P1 (SLA2013-490) | p.Pro34Ser | 69 | Female | Bulbar | sALS | UMN + LMN | 18 months | ex2 | Mutation |
| P2 (613-SN) | p.Pro34Ser | 57 | Male | Spinal | sALS** | UMN + LMN | 82 months* | ex2 | Mutation |
| P3 (SLA2012-251) | p.Pro34Ser | 44 | Female | Bulbar | sALS | UMN + LMN | 15 months | ex2 | Mutation |

* alive;

** see text for details.

sALS, sporadic ALS; fALS, familial ALS; UMN, upper motor neuron; LMN, lower motor neuron