Neurobiology of Aging xxx (2015) 1–3

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

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

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ARTICLE INFO

Article history:
Received 23 November 2014
Received in revised form 20 January 2015
Accepted 21 January 2015

Keywords:
Amyotrophic lateral sclerosis
Familial
Sporadic
CHCHD10

ABSTRACT

Mutations in *CHCHD10* have recently been described as a cause of frontotemporal dementia (FTD) comorbid 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 2 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 subjects without dementia or other atypical clinical signs.

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[1] The members of ITALSGEN Consortium are listed in the Acknowledgment section.

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http://dx.doi.org/10.1016/j.neurobiolaging.2015.01.017

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 3–5 years of symptom onset. Approximately 10% of patients have a family history of 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., 2012; Renton et al., 2014).

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

2. Methods

2.1. Samples

The following were included in the sample: (1) 64 unrelated Italian probands with fALS recruited through the Italian ALS Genetic (ITALSGEN) consortium; (2) 224 apparently sALS Italian cases 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 (Chio et al., 2012); and (3) 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 sALS 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) (Montuschi et al., 2014; Strong et al., 2009).

2.2. Sequencing of CHCHD10

Coding exons and flanking intronic regions of *CHCHD10* (NM_213720.2) were amplified by polymerase chain reaction (PCR) and analyzed by denaturing high-performance liquid chromatography (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 provided 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 7 cases carrying 4 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 3 apparently sALS 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). These variants were of unclear pathogenicity as they were also present in Italian controls and 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. Neurologic examination performed 6 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 years of age from lung cancer and her mother at 61 years because of breast cancer. Her 2 siblings were negative for neurologic 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. Neurologic 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 magnetic resonance imaging was normal, and neurophysiological examination demonstrated chronic denervation of cervical region. He was cognitively normal. Family history was negative for ALS. However, his 94-year-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. Neurologic examinations performed 3 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 because of cirrhosis and her mother died at 56 years of age because of cerebral hemorrhage. Her 6 siblings were negative for neurologic disorders. She died from respiratory failure 15 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

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)	—

Key: fALS, familial amyotrophic lateral sclerosis; FTD, frontotemporal dementia; sALS, sporadic amyotrophic lateral sclerosis.