LETTER TO THE EDITOR

CHCHD10 mutations in Italian patients with sporadic amyotrophic lateral sclerosis

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Sir,

We read with interest the paper recently published in Brain (Bannwarth et al., 2014) reporting a mutation in CHCHD10 (c.176C > T, p.Ser59Leu) in familial amyotrophic lateral sclerosis (ALS) with frontotemporal dementia (FTD). Interestingly, the mutated patients also showed signs of muscle mitochondrial pathology consisting of cytochrome c oxidase (COX)-negative fibres, ultrastructural mitochondrial abnormalities, impaired respiratory chain activity, and altered mitochondrial DNA (mtDNA) maintenance (multiple deletions).

Additional CHCHD10 mutations were reported by Müller et al. (2014) who identified the c.44C > A variant (p.Arg15Leu) in two German familial ALS cases and the variant c.197C > A (p.Gly66Val) in a Finnish patient with familial motor neuron disease with predominant lower motor neuron involvement. Chaussenot et al. (2014) screened CHCHD10 in a cohort of 80 French patients with sporadic FTD-ALS, disclosing the p.Pro34Ser mutation in two independent subjects. Mutated patients also featured sensorineural hypoacusia typically associated with mitochondrial disease, but mitochondrial dysfunction was not formally documented. Finally Johnson et al. (2014) investigated 85 independent North American cases with familial ALS, reporting the p.Arg15Leu mutation in three of them.

Here we report clinical, biochemical, and molecular findings of an Italian patient affected by sporadic early-onset ALS and muscle mitochondrial pathology associated with a novel CHCHD10 mutation. Moreover we investigated a cohort of Italian sporadic ALS patients, supporting the modest, but not negligible causative role of CHCHD10 variations.

We previously found severe histochemical COX deficiency in 7 of 50 muscle biopsies from patients with sporadic ALS (Crugnola et al., 2010). Sequence analysis of ALS-related genes was negative in all patients but two (SOD1: p.Gln22Arg and TDP43: p.Ala382Thr). We sequenced CHCHD10 coding regions in the remaining five patients, disclosing the novel heterozygous transition c.239C > T in exon 2, resulting in the amino acid change p.Pro80Leu, in one of them (Fig. 1A).

The variant was absent in available repositories including 1000 Genome and Exome Variant Server databases as well as in 286 Italian control chromosomes. The affected proline is conserved across species (Fig. 1B) and the substitution with a
leucine is predicted as pathogenic according MutationTaster, PMut and SIFT algorithm. At age 25, the patient developed widespread fasciculations and progressive weakness and wasting of the left hand muscles, subsequently spreading in the following 8 months to the proximal muscles of the ipsilateral upper limb and to the contralateral hand. A neurological examination performed 2 years after onset was compatible with a flail-arm syndrome with severe, symmetrical brachial diplegia, and brisk bilateral patellar and Achilles reflexes. Bulbar and lower limbs muscles were spared. Routine blood tests were unremarkable, with the exception of a mild elevation of creatine kinase serum levels (255 U/l, reference values: 20–195 U/l). Anti-ganglioside antibodies and CSF analysis were also normal. Electrophysiological studies showed both acute and chronic denervation signs in all muscles of the upper limbs, and a reduced amplitude of transcranial magnetic stimulation motor-evoked potentials in the four limbs. Brain and cervical MRI did not show any abnormalities. The patient is still alive 8 years after onset of symptoms, having developed a severe flaccid tetraparesis, requiring nocturnal non-invasive positive-pressure ventilation. Respiratory chain analysis performed on biopsied skeletal muscle revealed combined respiratory chain deficiency (Fig. 1C).
large-scale rearrangements of muscle-extracted DNA by long and quantitative PCR without any evidence of deletion or depletion. DNA samples from parents and relatives were not available for molecular studies.

Encouraged by this finding, we performed CHCHD10 screening in 217 patients with sporadic ALS, 11 of which (5.1%) also showing dementia or behavioural changes compatible with the FTD-ALS diagnosis. CHCHD10 sequence analysis in 16 familial ALS cases and one familial ALS-FTD case was negative.

We disclosed the same p.Pro80Leu mutation in a male patient who developed diffuse fasciculations in the four limbs at 59 years of age. After 6 months he showed bulbar involvement, clumsiness in distal upper limb movements, distal upper and lower limb hypotrophy and signs of upper motor neuron degeneration. Brain and spinal cord MRI were unremarkable while EEG performed at early stage of disease presented sharped waves in the posterior regions. EMG showed signs of chronic and acute neurogenic abnormalities, with no myopathic changes. Blood examination only showed increased creatine kinase (320 U/l) and cholesterol 238 (reference values: 125–200 mg/dl) levels. Familial history was negative for neurological disorders with the exception of the mother, diagnosed for Parkinson’s disease.

Furthermore, we disclosed the p.Pro34Ser mutation, previously found by Chaussenot et al. (2014), in a second patient of our series, a female who developed bulbar ALS at 75 years of age followed, 1 year later, by non-invasive ventilation and upper limb weakness. In the following 2 years, she required a wheelchair and percutaneous endoscopic gastrostomy. No other clinical information is available from members of her family.

Overall we report CHCHD10 mutations in three Italian patients featuring sporadic ALS/FTD-ALS. Our study supports the involvement of this gene in 1.4% of Italian patients with a diagnosis of probable or definite motor neuron disease. This figure is lower with respect to prevalence observed in other studies (2.3–3.5% of familial ALS and 2.6% of sporadic motor neuron patients) but higher in comparison to other genes involved in sporadic ALS, immediately following the contribution of C9orf72 and SOD1 (Renton et al., 2014).

It is hard to pinpoint cardinal features of CHCHD10-mutated patients. Main clinical presentation is a motor neuron disorder pure or accompanied with cerebellar deficits or signs of fronto-temporal lobar degeneration, in two cases preceding the motor deficit. Disease onset ranges from 27–78 years of age. Bulbar symptoms are often observed at early stages of the disease. Clinical features may differ even in presence of the same mutations. Indeed, our two patients harbouring the same p.Pro80Leu change show variability in age (28 versus 59) and phenotype at onset (flail arm versus bulbar ALS). Similarly, the p.Pro34Ser mutation was found in two late-onset FTD-ALS French patients (Chaussenot et al., 2014) and gave rise to an almost pure motor neuron disease with a delay of 10 years later in our patient.

This heterogeneous landscape is further complicated by the recent report of Ajroud-Driss et al. (2014) who disclosed two CHCHD10 mutations in cis (namely, p.Arg15Ser and p.Gly58Arg) in affected members of a Puerto Rican multi-generational family with a pure early-onset mitochondrial myopathy, previously described by Heiman-Patterson et al. (1997). Muscle biopsy revealed the classical stigmata of mitochondrial involvement including ragged-red fibres, mitochondrial cristae alteration and severe respiratory chain deficiency. No signs of motor neuron pathology or dementia had been observed in mutated subjects, although the oldest patient investigated was 63 years old and no clinical update was provided. Remarkably, we previously documented muscle respiratory chain deficiency and multiple mtDNA deletions in a family with childhood-onset mitochondrial myopathy due to mutations in GFER, encoding a key protein of the disulphide relay system (Di Fonzo et al., 2009). This mechanism is used to import other members of the twin cysteine-x9-cysteine protein family (to which CHCHD10 belongs) into the mitochondrial intermembrane space where they participate in the assembly of respiratory chain complexes (Longen et al., 2009).

All of the CHCHD10 mutations so far described lie in exon 2 and affect conserved residues in different regions of the encoded protein (Fig. 1A). Sequencing exon 2 could be a rapid and cost-effective option for a preliminary screening, although we cannot exclude the involvement of other exons. Mutations seem to act according to a gain-of-function mechanism, but functional studies on most of them are not conclusive. Indeed, even the partial knock-down (40–50%) of CHCHD10 transcript in HeLa cells by siRNA transfection resulted in reduced ATP content and COX activity (Martherus et al., 2010).

CHCHD10 protein is ubiquitously expressed and particularly abundant in energy-demanding tissues such as the heart, skeletal muscle and liver. The protein is also present in brain extracts but specific subregions of the CNS, including the spinal cord, have not been addressed.

Motor neuron pathology has been rarely described in patients displaying mutations in genes encoding for mitochondrial proteins (Hirano et al., 2008; Ronchi et al., 2012). In contrast, several lines of evidence indicate that mitochondrial dysfunction underlines pathogenesis of sporadic and genetic forms of ALS (Cozzolino et al., 2013).

The discovery of CHCHD10 mutations in familial and sporadic cases of ALS/FTD-ALS reinforces the hypothesis that homeostasis of mitochondrial distribution is critical for survival of cortical and spinal motor neurons. The screening for CHCHD10 mutations in other cohorts of patients with pure or complex motor neuron disease is needed to understand the relevance of this mechanism.
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