

## LETTER TO THE EDITOR

### Two novel mutations in conserved codons indicate that *CHCHD10* is a gene associated with motor neuron disease

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Sir,

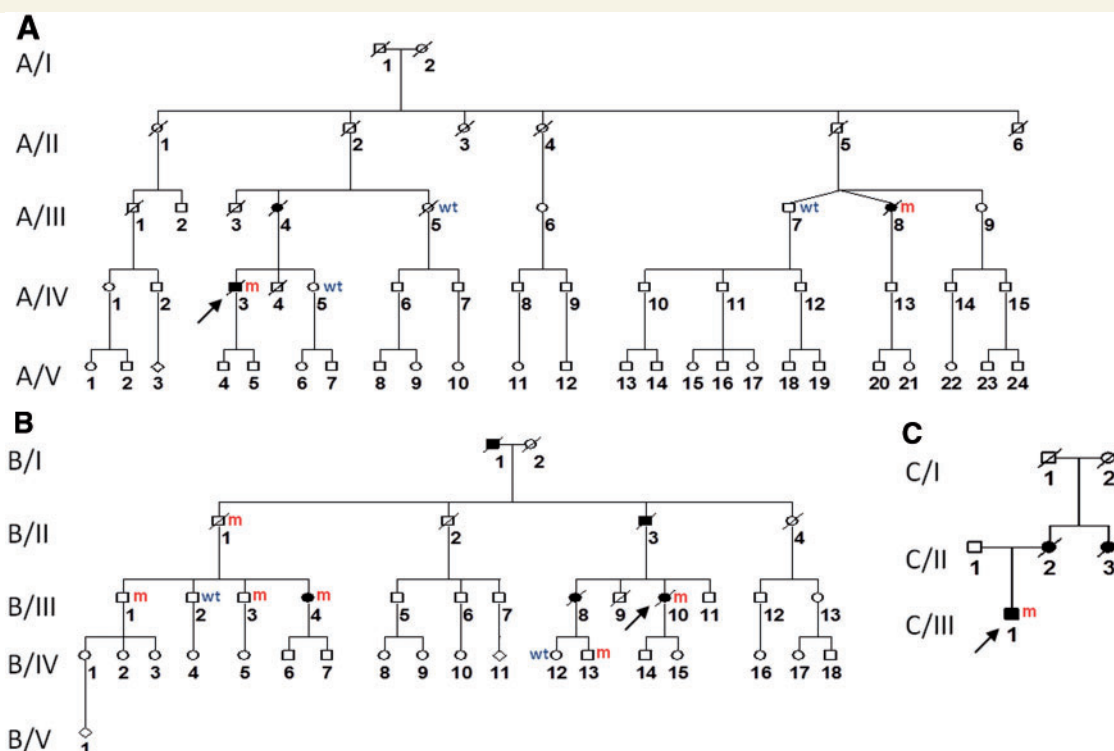
Recently, Bannwarth *et al.* (2014) reported a family with a mitochondrial DNA instability disorder that variably presented with cerebellar ataxia and myopathy but also with an ALS-FTD-like phenotype. A mutation in *CHCHD10*, which codes for the mitochondrially located coiled-coil-helix-coiled-coil-helix domain-containing protein 10, was identified by whole exome sequencing as the likely cause of disease in this family. Conceptually, this is an interesting finding, as it shows for the first time that mutation of a gene coding for a mitochondrial protein and subsequent mitochondrial dysfunction can be a primary cause of amyotrophic lateral sclerosis (ALS). To date, single nucleotide polymorphisms in the gene for the regulator of mitochondrial biogenesis PGC-1 $\alpha$  were described to have a disease-modifying effect in ALS (Eschbach *et al.*, 2013).

The most important genetic evidence for the causality of mutant *CHCHD10* presented by Bannwarth *et al.* is a p.Ser59Leu variant in this gene co-segregating with disease in one family. Moreover, the same variant was found in a patient of another ALS-FTD family. Lack of further DNA samples precluded segregation analysis in this family. Co-segregation in several families is desirable to

firmly establish a novel disease gene. We are therefore pleased to present here additional genetic support for *CHCHD10* as a novel ALS gene, and extend the phenotype/genotype correlation of *CHCHD10*-related neurodegeneration.

We have previously performed whole exome sequencing of DNA from 102 German and 26 Nordic (22 Swedish and four Finnish) familial ALS index patients where both a repeat expansion in *C9orf72* and mutations in *SOD1* had been excluded (Hübers *et al.*, 2014, and unpublished data). Sequencing was performed as 100 bp paired-end reads on HiSeq2000/2500 systems (Illumina). We generated on average 10 gigabases of sequence resulting in an average depth of coverage of 125 with 95% of the target regions covered at least 20 times.

Screening for *CHCHD10* mutations revealed a heterozygous c.44C>A variant (p.Arg15Leu) in two German index patients (Families A and B; Fig. 1A and B), and another heterozygous variant (c.197C>A; p.Gly66Val) in a Finnish patient with familial motor neuron disease (Family C; Fig. 1C). The patients had previously been screened negative for mutations in all known ALS genes (Andersen and Al-Chalabi, 2011; Renton *et al.*, 2014). Both variants were absent in the 1000 Genomes Project data



**Figure 1** Pedigrees of Families A, B and C. Arrows indicate the index patient. Genotypes of available DNA samples for the respective *CHCHD10* mutation are shown. wt = wild-type; m = mutant. DNA was not available from the affected Patient B/III.8 for genetic testing, but presence of the mutation in her son (Patient B/IV.13) indicates that she was an obligate p.Arg15Leu carrier. Filled symbols represent affected individuals.

( $n = 1700$ ), the Exome Variant Server (EVS) data that covers ~6500 individuals of European American and African American origin, as well as in 1000 exome sequence data of individuals without neurological disease, of predominantly German origin in our in-house database.

Both mutations affect amino acid residues that are highly conserved throughout mammalian species. Sanger sequencing of DNA from further family members, in addition to the index patients, revealed the segregation of the p.Arg15Leu variant in affected grand-cousins and cousins in Families A and B, respectively (Fig. 1A and B). No DNA was available from the deceased Patient B/III.8 in Family B, but presence of the variant in her son (Patient B/IV.13) indicates that she was an obligate p.Arg15Leu carrier. This variant was not found in five unaffected first degree relatives of ALS patients in the two families (Fig. 1), but could be identified in one unaffected obligate mutation carrier and two presently unaffected siblings of Patient B/III.4 in Family B (Fig. 1B). This observation is in-line with both families' pedigrees, which suggested *a priori* an autosomal-dominant mode of inheritance with incomplete penetrance, based on unaffected individuals transmitting the disease (Fig. 1A and B), and is compatible with what has been found in pedigrees with other ALS genes (Andersen *et al.*, 1997). The mutation of the N-terminal Arg15 in *CHCHD10* is located within a potential N-terminal mitochondrial targeting sequence (<http://www.uniprot.org/uniprot/Q8WYQ3>). Taking together its absence in reference databases, co-segregation with disease in two families and the high

evolutionary conservation, we propose that p.Arg15Leu in *CHCHD10* is most likely the causative mutation in these two families with motor neuron disease.

Unavailability of DNA samples precluded co-segregation analysis of our second novel variant p.Gly66Val from the Finnish index patient (Family C; Fig. 1C). The patient had slowly ascending progressive motor neuron disease, as did his mother and aunt. Interestingly, the p.Gly66Val is highly reminiscent of the nearby motor neuron disease mutation in *CHCHD10* described recently (p.Ser59Leu) (Bannwarth *et al.*, 2014), in that it also affects one of the few polar amino acids in the N-terminal hydrophobic  $\alpha$ -helix of *CHCHD10*. Hence the absence in a total of 9200 reference exome sequence data sets, high evolutionary conservation of Gly66 in *CHCHD10* (including non-mammalian vertebrates) as well as the obvious similarity with the motor neuron disease mutation reported previously are at least suggestive of the Gly66Val amino acid change in *CHCHD10* being the causative mutation in this family.

In contrast to some of the *CHCHD10* mutant patients reported recently (Bannwarth *et al.*, 2014), our patients did not present with cerebellar deficits or signs of fronto-temporo-lobe degeneration. All were diagnosed with motor neuron disease in a specialized ALS centre, and presented with progressive, ascending, asymmetrical and mostly flaccid paresis and muscle wasting with lower limb onset in the Finnish patient (p.Gly66Val mutation) or exclusively upper limb onset in seven German patients (p.Arg15Leu mutation), for whom respective records were

available. Age of disease onset ranged from 35 to 73 years ( $46.7 \pm 11.8$  years; mean  $\pm$  SD). In addition to the clinically predominant lower motor neuron involvement, Patients A/IV.3, B/III.8 and B/III.10 (Fig. 1) had also asymmetrical hyper-reflexia and spasticity. Patients A/III.4, A/III.8, B/III.4, B/III.8 and B/III.10 (Fig. 1) developed bulbar symptoms during the course of disease, and Patient A/III.8 received a percutaneous endoscopic gastrostomy due to severe dysphagia. All examinations, including cranial and cervical MRI, CSF analysis, neurography, EMG or laboratory testing were in accordance with the diagnosis. Unfortunately, none of the patients had a muscle biopsy performed, but available EMG results from Patients A/IV.3, B/III.10 and C/III.1 report an acute and chronic neurogenic rather than a myopathic pattern. Interestingly, besides a predominant lower motor neuron affection with spinal onset, slow disease progression and long survival times seem to be another common clinical feature of the majority of *CHCHD10* mutant motor neuron patients reported to date by us and by Bannwarth *et al.* (2014). Mean survival times of our patients (including Patient B/III.4 who is alive after a disease course of 15 years) range from 6 to 17 years ( $10.7 \pm 4.5$  years; mean  $\pm$  SD; survival times from seven patients available).

Our data thus represent strong support for *CHCHD10* being a novel motor neuron disease/ALS gene, providing important evidence complementary to the first description (Bannwarth *et al.*, 2014). *CHCHD10* mutations may be associated with a variable, but in most cases, slowly progressive phenotype with incomplete penetrance, at least with regard to the p.Arg15Leu mutation. The reduced penetrance should lead to screening for *CHCHD10* mutations also in seemingly sporadic motor neuron disease patients. Considering the specific mitochondrial localization and its probable role in the maintenance of mitochondrial integrity, *CHCHD10* points to a novel pathogenic principle in motor neuron disease.

## Funding

This work was supported in whole or in parts by grants from the German Federal Ministry of Education and Research (STRENGTH consortium/BMBF, 01GI0704; The German Network for ALS Research (MND-NET)), the Charcot Foundation for ALS Research, the Swabian ALS Registry, the virtual Helmholtz Institute “RNA-Dysmetabolismus bei ALS und FTD”, the Knut and Alice Wallenberg Foundation, The Bertil Hållsten and Brain Research Foundation, The Ulla-Carin Lindquist ALS Research Foundation, The Swedish Research Council and the Swedish Brain Power.

## References

- Andersen PM, Al-Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: what do we really know? *Nat Rev Neurol* 2011; 7: 603–15.
- Andersen PM, Nilsson P, Keranen ML, Forsgren L, Hagglund J, Karlsborg M, *et al.* Phenotypic heterogeneity in motor neuron disease patients with CuZn-superoxide dismutase mutations in Scandinavia. *Brain* 1997; 120 (Pt 10): 1723–37.
- Bannwarth S, Ait-El-Mkadem S, Chaussonot A, Genin EC, Lacas-Gervais S, Fragaki K, *et al.* A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through *CHCHD10* involvement. *Brain* 2014; 137: 2329–45.
- Eschbach J, Schwalenstöcker B, Soyal SM, Bayer H, Wiesner D, Akimoto C, *et al.* PGC-1alpha is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis. *Hum Mol Genet* 2013; 22: 3477–84.
- Hübers A, Marroquin N, Schmoll B, Vielhaber S, Just M, Mayer B, *et al.* Polymerase chain reaction and Southern blot-based analysis of the C9orf72 hexanucleotide repeat in different motor neuron diseases. *Neurobiol Aging* 2014; 35: 1214.e1–6.
- Renton AE, Chio A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 2014; 17: 17–23.