

LETTER TO THE EDITOR

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

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Sir,

In a recent publication in *Brain*, we described a novel heterozygous *CHCHD10* mutation (c.176C>T; p.Ser59Leu) in a large French family with a late-onset phenotype including cognitive decline resembling frontotemporal dementia (FTD), motor neuron disease, cerebellar ataxia and mitochondrial myopathy with multiple mtDNA deletions (Bannwarth *et al.*, 2014). We found the same pathogenic mutation in one family in a cohort of 21 families with pathologically proven FTD-ALS (amyotrophic lateral sclerosis). We also showed that the *CHCHD10* gene encodes a mitochondrial protein located in the intermembrane space, and that it is likely involved in the maintenance of cristae junctions and mtDNA stability. This work led to the identification of a novel gene responsible for FTD-ALS and the intriguing realization that mitochondrial dysfunction could be an

important pathophysiological player contributing to the aetiology of these diseases.

The letter from Müller *et al.* (2014) contains important information that confirms the involvement of the *CHCHD10* gene in ALS. They performed whole exome sequencing in 102 German and 26 Nordic ALS patients. By screening *CHCHD10*, they identified two novel heterozygous variants: c.44G>T (p.Arg15Leu) in two German families and c.197G>T (p.Gly66Val) in one Finnish family. Although it was not possible to confirm the deleterious nature of these variants through segregation analysis due to the limited number of DNA samples available per family, their absence in public and in-house SNP databases, and in ethnically matched controls in addition to the evolutionary conservation of the corresponding amino acid residues are all in favour of a pathogenic effect.

The p.Arg15Leu substitution is located in a non-structured N-terminal region whereas the p.Gly66Val substitution is located in the hydrophobic N-terminal α -helix. The latter amino acid change is in close physical proximity to the c.176C>T (p.Ser59Leu) mutation that we previously reported in FTD-ALS, and might possibly destabilize the α -helix conformation with resulting altered protein-protein interactions (Banci *et al.*, 2012; Bannwarth *et al.*, 2014).

In our two families with FTD-ALS and *CHCHD10* mutations (Bannwarth *et al.*, 2014), the age at onset ranged from 49 to 65 years (55.5 ± 5.9 years; mean \pm SD) and was higher than in the three ALS families reported by Müller and colleagues, but this is not surprising given that FTD-ALS has a later onset compared with ALS. Regarding clinical presentation, bulbar palsy with dysarthria and dysphagia was found predominant and/or at disease onset in 6 out of 8 patients from our original large French family (Bannwarth *et al.*, 2014). The additional index case identified from our cohort of 21 families with FTD-ALS, carrying the same c.176C>T (p.Ser59Leu) mutation, also had a predominant pseudobulbar syndrome (Bannwarth *et al.*, 2014). In the study by Müller *et al.*, several patients from the two German families carrying the c.44G>T (p.Arg15Leu) mutation developed bulbar symptoms during the course of their disease. However, the clinical presentation was typical of classical motor neuron disease with paresis, muscle wasting and spasticity but without predominant bulbar syndrome. None of these patients had a cerebellar ataxia that was observed in several affected individuals from our original large French family, even in the absence of motor neuron disease. We agree with these authors that *CHCHD10* mutations might be responsible for a slower disease progression and longer survival times. In our original large French family, several patients survived more than 10 years after being first diagnosed. Only one individual (Patient V-2), who presented with pure 'ALS-like' motor neuron disease, died 1 year after the onset of symptoms. The index case from our French cohort of FTD-ALS families had been symptomatic for 8 years before he was lost from follow-up, again pointing to a less aggressive neurological course. Finally, the ALS patients described by Müller *et al.* did not present what is generally regarded as classical features of mtDNA maintenance disorders, namely sensorineural deafness or ptosis. In the absence of muscle biopsy specimens, we cannot speculate whether the three new families reported have evidence of mtDNA instability in the form of multiple mtDNA deletions, but nevertheless, it is clear that the phenotypic spectrum associated with mtDNA maintenance disorders will continue to expand with the advent of next-generation whole exome sequencing.

It has been estimated that 15% of patients with FTD meet the diagnostic criteria for ALS and as many as 15% of patients with ALS develop FTD symptoms (Ling *et al.*, 2013). These diseases are now recognized as a continuum of a broad neurodegenerative disorder with overlapping symptoms. The notion of continuum was reinforced by the identification of common molecular players involving TDP-43, FUS/TEL, ubiquilin-2, VCP and *C9orf72* gene leading to the hypothesis that disruption of both RNA and protein homeostasis is a common pathogenic mechanism underlying ALS and FTD (for review see Ling *et al.*, 2013). Since the first reports of mitochondrial abnormalities in tissues from patients with ALS, mitochondrial dysfunction has emerged as an

important contributor to the evolution and progression of the disease (Affi *et al.*, 1966). The subsequent identification of mitochondrial dysfunction in patients and mouse models harbouring deleterious *SOD1* mutations has further reinforced this important pathological concept (Cozzolino *et al.*, 2013). It has been suggested that a defect in mitochondrial axonal transport contributes to motor neuron degeneration in ALS models (De Vos *et al.*, 2007). Targeted expression of *SOD1* in the mitochondrial intermembrane space rescues motor axon outgrowth, normalizes the mitochondrial redox state in *Sod1*^{-/-} neurons *in vitro*, and prevents weakness and denervation in *Sod1*^{-/-} mice (Fischer *et al.*, 2011). These data suggest that mitochondrial oxidative stress is an underlying cause of distal motor axonopathy, and demonstrate that localization of *SOD1* in the intermembrane space is sufficient for the survival of motor axons (Fischer *et al.*, 2011). However, until now, it had never been proven that mitochondrial dysfunction might be at the origin of FTD-ALS phenotypes. The identification of a novel pathway involving *CHCHD10* is therefore a fundamental step in understanding how disruption of both RNA and protein homeostasis and/or mitochondrial dysfunction can lead to the same disease.

In conclusion, the data reported by Müller *et al.* confirm the clinical, molecular and mechanistic continuum between FTD and ALS by demonstrating the involvement of *CHCHD10* mutations in familial pure ALS. As this gene was involved in 3 out of 128 patients (2.3%) in their study, *CHCHD10* mutations are not likely a common cause of ALS in German and Finnish populations. However, this result is similar to the one we report in a recent study that found 2.6% of patients carrying a *CHCHD10* mutation in a French cohort of 115 individuals with FTD and FTD-ALS phenotypes (Chausseot *et al.*, 2014). Further studies in larger cohorts are needed to determine the frequency of *CHCHD10* mutations in sporadic and familial ALS from different geographical backgrounds. The cellular pathways that are disrupted by the aberrant *CHCHD10* mutant protein also need to be elucidated and this will undoubtedly throw more interesting insight into the role of mitochondrial dysfunction in neurodegeneration.

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