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The *CHCHD10* P34S variant is not associated with ALS in a UK cohort of familial and sporadic patients

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Abstract

Mutations in *CHCHD10* have recently been reported as a cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). To address the genetic contribution of *CHCHD10* to ALS we have screened a cohort of 425 UK ALS+/-FTD patients and 576 local controls in all coding exons of *CHCHD10* by Sanger sequencing. We identified a previously reported Pro34Ser variant that was also present in neurologically healthy controls ($p=0.58$). Our results suggest that *CHCHD10* is not a primary cause of ALS in UK cases.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by lower and upper motor neuron degeneration. Approximately 5-10% of ALS cases are familial (FALS) with the remainder presenting in a sporadic manner (SALS). Mutations in four major genes, *SOD1*, *TARDBP*, *FUS* and the intronic *C9orf72* GGGGCC expansion account for approximately 50% of FALS. A recent report by Bannwarth et al. (2014) described a novel p.Ser59Leu (c.176C>T) mutation in *CHCHD10* accounting for ALS and frontotemporal dementia (FTD). Subsequent independent screenings have also confirmed a putative role of *CHCHD10* in a broad range of neurodegenerative

diseases (Auranen et al., 2015; Johnson et al., 2014; Kurzwelly et al., 2015; Penttila et al., 2015; Zhang et al., 2015) (**Supplementary Table 1**). Here we report the first mutation screening of *CHCHD10* in a UK cohort of familial and sporadic patients.

2. Methods

All 4 coding exons of *CHCHD10* (NM_213720.2) were amplified by PCR and products directly sequenced with an ABI3130 (Applied Biosystems, Warrington, UK) (**Supplementary Information**).

3. Results

Analysis of an existing in-house cohort of UK FALS exomes revealed poor coverage (only 24.8% of samples met the minimum read depth ie. 8x) across exon 2 of *CHCHD10* that is a hotspot for reported mutations. We therefore Sanger sequenced all exons of *CHCHD10* in 163 FALS, 262 SALS and 576 healthy controls to ensure complete coverage of the gene. We identified the previously described p.Pro34Ser (c.100C>T) variant in 1 FALS (0.61%), 4 SALS (1.53%) and 8 controls (1.39%). One of the sporadic cases also carried the *C9orf72* hexanucleotide expansion. Further SNP genotyping of position 100 in additional UK controls detected the minor allele, thymine, in 3/640 (0.31%) individuals. In line with Zhang et al. (2015) and Dobson-Stone et al. (2015), our screening results identified that the P34S frequency was non-significant in ALS cases versus controls (ALS/FTD n=5/425, 1.2% vs controls n=11/1,216, 0.82%) ($p=0.58$, 2-tailed Fischer's exact test). Additionally, several other *CHCHD10* variants were identified, however none were predicted to be pathogenic as they were either found only in controls, or were insufficiently rare in ExAC (**Supplementary Table 2**). None of the changes were predicted by Netgene2 (<http://www.cbs.dtu.dk/services/NetGene2/>) to alter splicing.

4. Discussion

Our study failed to identify any disease-relevant variants, suggesting that mutations in *CHCHD10* are not a common cause in UK ALS/FTD patients. The Pro34Ser variant was initially identified in two unrelated French FTD-ALS individuals (Chausseot et al., 2014) and one Italian sporadic case (Ronchi et al., 2015). However in an Australian cohort of FTD and/or dementia patients (n=370), seven cases as well as nine aged controls (n=807) were found to carry the Pro34Ser change, suggesting that it is non-contributory to disease ($p=0.290$) (Dobson-Stone et al., 2015). Although this variant is absent in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>) and EVS (<http://evs.gs.washington.edu/EVS/>), it is found nine times in the Non-Finnish European subset of the Exome Aggregation Consortium data at a frequency of 0.60% (n=1,508) (ExAC, <http://exac.broadinstitute.org/>). It is worth

noting that the coverage of exon 2 at a read depth of at least 10x is relatively low (47.10%) with only 8.88% of samples achieving this threshold at Pro34 (ExAC release 1) potentially due to the GC-rich nature of this region (77.3% GC-content). The low coverage from next generation sequencing suggests that the true frequency of variants in controls may indeed be higher, as reflected by our Sanger sequencing data. Low coverage of exome sequencing reads from public databases is a note of caution when interpreting newly identified variants.

To date, there has been an absence of published *in-vitro* studies investigating the functional impact of ALS-associated *CHCHD10* mutations with the exception of the Ser59Leu change. Based on impact prediction tools and conservation of the codon, the Pro34Ser variant is predicted to influence the protein's stability and/or protein-protein interactions (Chausselet et al., 2014). One sporadic case from our cohort carried both a *C9orf72* hexanucleotide repeat expansion and a Pro34Ser change as seen by Dobson-Stone et al. (2015). Several studies have reported double mutations in ALS or FTD associated genes, suggesting an oligogenic model requiring multiple 'hits' to manifest disease (King et al., 2013; van Blitterswijk et al., 2013; van Blitterswijk et al., 2012). Together, these results suggest that the Pro34Ser variant is not pathogenic as the primary cause of disease, and its potential role as a susceptibility allele may be questionable. Functional investigation is essential to critically assess the contribution of *CHCHD10* as a causative gene in ALS pathobiology as well as other neurodegenerative phenotypes.

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Conflicts of interest: none