LETTER TO THE EDITOR

Is CHCHD10 Pro34Ser pathogenic for frontotemporal dementia and amyotrophic lateral sclerosis?

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Sir,

We read with interest the recent publication in Brain by Bannwarth et al. (2014a) identifying CHCHD10 mutations in patients with frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS). Subsequent reports have identified CHCHD10 variants in other FTD-ALS patients (Chaussenot et al., 2014), as well as those with pure ALS (Johnson et al., 2014; Muller et al., 2014; Ronchi et al., 2014), mitochondrial myopathy (Ajroud-Driss et al., 2015) and spinal motor neuronopathy (Penttila et al., 2015). Seven different amino acid substitutions have been identified, all arising in exon 2, and the prevalence of CHCHD10 variants is estimated at 1.4–3.5% in ALS or FTD-ALS spectrum cases of European ancestry. Here we present several strands of evidence, suggesting that not all CHCHD10 missense variants are pathogenic.

In the present study, we performed whole-exome sequencing of 16 unrelated patients with FTD with a family history of dementia or ALS and who did not have mutations in known dementia or ALS genes. The ethical review boards at each institution approved the study, and informed consent was obtained from each participant or their next of kin. Exome-enriched DNA samples were subjected to 100 bp paired-end sequencing on the Illumina HiSeq2000 with 50 x coverage (performed by Macrogen). Variants were called using Genome Analysis Toolkit (GATK) best practices. This identified a missense variant in CHCHD10, c.100C>T (p.Pro34Ser, NM_213720.2), in Proband 2. This variant was reported previously in two unrelated French patients with FTD-ALS (Chaussenot et al., 2014) and one Italian ALS patient (Ronchi et al., 2015). Visual examination of our whole-exome sequencing reads revealed that proband 1 also potentially carried this variant, which had likely been overlooked by GATK variant calling as it was present on only one of eight reads covering the region (Fig. 1A). This variant was not present in dbSNP (http://www.ncbi.nlm.nih.gov/snp/) or control individuals from the 1000 Genomes Project (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) or the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/).

Proband 1 presented at age 62 years with word-finding difficulties and clumsiness. She subsequently became...
increasingly dysphasic and a SPECT scan was compatible with a diagnosis of FTD. Her mother, maternal grandmother and a maternal cousin were reported to have ALS. Proband 2 presented at age 71 with decreased motivation, personality change, concreteness and lack of insight and a PET scan showed frontal hypometabolism. She died at age 79 and neuropathology showed severe frontotemporal atrophy with histological diagnoses of Alzheimer’s disease, Parkinson’s disease and congophilic angiopathy. Two sisters also developed dementia in their late sixties/early seventies and their mother had late-onset dementia. Sanger sequencing confirmed the presence of the Pro34Ser variant in both probands, but revealed that the variant did not segregate with disease in Family 2, with only one of the two affected sisters carrying the variant. No DNA samples were available from the family members of Proband 1.

We subsequently screened CHCHD10 exon 2 by Sanger sequencing of an Australian cohort comprising 132 individuals with early-onset dementia (51 familial, 81 no/unknown family history), including 15 subjects with repeat expansions in C9orf72. Five additional Pro34Ser carriers were identified: two patients with clinically ascertained FTD and no family history; one with neuropathologically confirmed frontotemporal lobar degeneration with TARDBP inclusions (FTLD-TDP) and three relatives with dementia; and two patients with clinically ascertained Alzheimer’s disease and multiple relatives with dementia. Of note, the Pro34Ser carrier with FTLD-TDP also harboured the C9orf72 repeat expansion that is causal of FTD and ALS (Patient 2 in Dobson-Stone et al., 2013).

We also screened CHCHD10 exon 2 in a second Australian cohort comprising 145 FTD cases (30 familial, 115 no/unknown family history) with precise clinical phenotyping (53 behavioural variant FTD, 27 semantic dementia, 20 progressive non-fluent aphasia, 5 progressive supranuclear palsy, 18 corticobasal syndrome, 22 FTD-ALS), including 22 subjects with known mutations (15 C9orf72, 4 GRN, 2 MAPT, 1 VCP). We did not identify any Pro34Ser carriers in this cohort. Given the disparity in allele frequency between our early-onset dementia and FTD cohorts, we considered the possibility that the Pro34Ser variant leads to a disorder that is similar to FTD but doesn’t conform to standardized criteria for FTD subtypes. We therefore performed Sanger sequencing of 77 samples who presented with an initial FTD diagnosis (14 familial, 63 no/unknown family history) but after detailed neuropsychological testing were diagnosed as having a different disorder (35 Alzheimer’s disease, 33 logopenic aphasia, two behavioural variant FTD phenocopy, two dementia with Lewy bodies, three posterior cortical atrophy, one
Parkinson’s disease, one vascular dementia) but no more carriers were identified.

To determine frequency of the Pro34Ser variant in the general population, we performed Sanger sequencing of 807 aged Australian Caucasian control subjects from the Sydney Memory and Ageing Study (Sachdev et al., 2010). Subjects underwent detailed neuropsychiatric and medical assessments at collection (mean age ± standard deviation at collection = 78.4 ± 4.7 years). All individuals with dementia at study commencement were excluded. Nine Pro34Ser carriers were identified in this cohort (allele frequency 9/1614, 0.56%). Sufficient cognitive data were available to determine mild cognitive impairment (MCI) status for 714 individuals, including all nine Pro34Ser carriers. Three carriers showed MCI at ages 75, 82 and 87, but the six other carriers were cognitively intact (at ages 72, 74, 80, 81, 84 and 85). This compares with an MCI diagnosis in 251/705 (35.6%) of non-carriers. No history of ALS was reported.

We also consulted the Exome Aggregation Consortium browser (http://exac.broadinstitute.org), a data set comprising whole exome data from 60706 unrelated individuals collected from healthy control and various disease cohorts, including individuals with schizophrenia, bipolar disorder and Tourette syndrome but no cohorts from neurodegenerative diseases. Consistent with our whole exome data, mean coverage at the 5’ end of CHCHD10 exon 2 was low, with <10% individuals sequenced showing over 10× coverage in the vicinity of the Pro34Ser substitution (Fig. 1B). Ten Pro34Ser carriers were reported in the database, giving a frequency of 1/328 African origin alleles (0.30%) and 9/3016 European origin alleles (0.30%). Examination of quality control data revealed that 87 Pro34Ser carriers had been detected, but the majority were not included in the reported allele count due to the low genotype quality (Fig. 1C).

CHCHD10 Pro34 is invariant in mammals, zebrafish, nematode and fruit fly but is serine in frog (Xenopus tropicalis). Eight programs were used to predict pathogenicity of the Pro34Ser variant: PolyPhen-2, SIFT, LRT, FATHMM, MutationAssessor, MutationTaster (all accessed through annotation database dbNSFP; Liu et al., 2013), PON-P2 (http://structure.bmc.lu.se/PON-P2/) and Sibyl (http://bioinformatics.ua.pt/sibyl/). Six of these (PolyPhen-2, SIFT, FATHMM, MutationAssessor, PON-P2, Sibyl) predicted that this substitution was not deleterious.

In summary, our study has demonstrated for the CHCHD10 Pro34Ser variant: (i) non-segregation with disease in FTD Family 2; (ii) presence in an FTD patient who harbours another established pathogenic mutation; (iii) presence in 9/807 non-demented aged controls, six of whom were verified to be cognitively intact in their 70s–80s; (iv) predicted non-pathogenicity in six of eight functional prediction programs. None of these observations in isolation would be sufficient evidence to discount the pathogenicity of this variant. It is possible that the Pro34Ser-negative sister in FTD Family 2 is a phenocopy, especially given the relatively late onset of disease, which would explain apparent non-segregation in this family. Only the proband underwent a full clinical assessment, but we note that the phenotype of the Pro34Ser-negative individual (significant memory impairment with no frontal features) was different to her sisters. With reference to point (iii), FTD patients with more than one bona fide pathogenic mutation have been described before (van Blitterswijk et al., 2013). However, the convergence of all four strands of evidence indicates that Pro34Ser is not a disease-causing mutation. We note, as have others (Bannwarth et al., 2014b; Johnson et al., 2014), that this region of CHCHD10 is refractory to whole-exome enrichment. This means that genuine carriers of Pro34Ser and other variants in the 5’ region of CHCHD10 exon 2 are likely to be missed using this technique. We suspect that this is not an isolated phenomenon and thus the apparent absence or low frequency of any putative disease-causing variant in whole-exome sequencing databases should not be solely relied upon to deduce its pathogenicity.

We did observe a nominally significant difference in Pro34Ser allele frequency between our aged controls (9/1614) and our early-onset dementia cohort (5/264, Fisher’s exact test \( P = 0.036 \)) but not in all dementia cohorts combined (7/740, \( P = 0.290 \)). Larger-scale association studies would have to be performed to determine whether Pro34Ser is an FTD/ALS risk allele with low penetrance. Bannwarth et al. (2014c) reported that functional tests on reported CHCHD10 variants (including Pro34Ser) indicate a biologically plausible link with neurodegeneration, although these data are at present unpublished. Such functional studies will provide critical evidence to determine whether Pro34Ser and other CHCHD10 variants do in fact play a role in disease risk.

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References


