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LETTER TO THE EDITOR

Screening for *CHCHD10* mutations in a large cohort of sporadic ALS patients: no evidence for pathogenicity of the p.P34S variant

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Sir,

Bannwarth *et al.* (2014) reported a mutation in *CHCHD10* responsible for a variable phenotype including cerebellar ataxia, hearing impairment, myopathy, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This gene encodes the coiled-coil helix coiled-coil helix domain-containing protein 10 of largely unknown function. It is a strictly mitochondrially located protein that may be involved in respiratory chain function or mitochondrial genome stability (Bannwarth *et al.*, 2014). Several subsequent publications provided further evidence that *CHCHD10* mutations can cause familial motor neuron disease/ALS (familial ALS), with an unusually slow disease progression in most patients (Bannwarth *et al.*, 2014; Johnson *et al.*, 2014; Müller *et al.*, 2014; Kurzwelly *et al.*, 2015; Penttila *et al.*, 2015). As an important argument for *CHCHD10* causing ALS, co-segregation with disease could be demonstrated for the three *CHCHD10* variants p.R15L (Johnson *et al.*, 2014; Müller *et al.*, 2014; Kurzwelly *et al.*, 2015), p.S59L (Bannwarth *et al.*, 2014) and p.G66V (Penttila *et al.*, 2015). The strongest genetic evidence for causality exists with regard to the p.G66V mutation, which was first detected by Müller *et al.* (2014) in a single patient with familial ALS and subsequently shown to co-segregate with a slowly progressing motor neuron disease in a total of 17 pedigrees (Penttila *et al.*, 2015). Hence there is meanwhile unequivocal evidence that *CHCHD10* mutation can cause familial motor neuron degeneration. No association of *CHCHD10* variants with ALS could be shown in genome-wide association studies, which can plausibly be explained by the low frequency of *CHCHD10* mutations in familial ALS patient cohorts (Bannwarth *et al.*, 2014; Johnson *et al.*, 2014; Müller *et al.*, 2014). Consequently, due to the lack of co-segregation data, the contribution of *CHCHD10* mutations to the pathogenesis of sporadic ALS is less evident, although rare missense variants in this gene have been detected by screening of smaller cohorts of sporadic ALS patients (Chaussenot *et al.*, 2014; Chio *et al.*, 2015; Ronchi *et al.*, 2015). Arguments for causality of *CHCHD10* variants in sporadic ALS are restricted to their absence in publicly accessible whole exome sequence databases, evolutionary conservation of altered amino acid residues and bioinformatic prediction of damaging effects.

In order to further define the contribution of *CHCHD10* mutations to sporadic ALS we screened a cohort of 355 unrelated ALS patients with a negative family history regarding the ALS/FTD disease spectrum. The whole *CHCHD10* protein coding sequence was Sanger sequenced, and respective primer sequences are available on request. We found the heterozygous *CHCHD10* missense variant p.P34S (c.100C>T) in four patients with sporadic ALS. No other *CHCHD10* variants were observed.

The p.P34S *CHCHD10* variant has been reported in ALS patients previously. It was described by four different sporadic ALS or FTD studies (Chaussenot *et al.*, 2014; Chio *et al.*, 2015; Dobson-Stone *et al.*, 2015; Ronchi *et al.*, 2015). The variant was mostly considered pathogenic, based on the conserved amino acid change and absence of the variant in the exome variant server (EVS; http://evs.gs.washington.edu/EVS/) as well as up to 286 controls matched for the ethnic and geographical background of the respective patient cohorts. However, in the most recent study the variant was observed in 7 out of 370 (1.9%) dementia/FTD cases, but also in 9 out of 807 (1.1%) geographically matched controls. In addition, non-segregation in one FTD family was reported, questioning whether the p.P34S variant in *CHCHD10* is disease-causing (Dobson-Stone *et al.*, 2015).

We compared the frequency of p.P34S in our German sporadic ALS cohort (1.1%) to in-house exome sequence data sets of individuals of German origin without neurological disease. Sequencing was performed as 100 bp paired-end reads on HiSeq2000/2500 systems (Illumina). We included 393 individuals in which the p.P34/c.100C position in *CHCHD10* was covered with a read depth of at least 10-fold. We observed the p.P34S/c.100C>T variant in 6 out of these 393 controls (1.5%), thus at a comparable and nominally even higher frequency than in the case cohort. As described before (Johnson *et al.*, 2014; Dobson-Stone *et al.*, 2015) mean coverage at the 5' end of the *CHCHD10* exon 2 by whole exome sequencing was poor, thus some *CHCHD10* p.P34S carriers could have escaped the detection and their frequency might even be higher in controls than apparent in our analysis. In addition, differently than stated earlier (Chio *et al.*, 2015), but mentioned in the most recent report on p.P34S in FTD/ALS (Dobson-Stone *et al.*, 2015), the heterozygous p.P34S variant is found in 9 out of 1499 European individuals in the ExAC data set (http://exac.broadinstitute.org; 13 April 2015) in which the respective position is covered by whole exome sequencing (0.6%). The nominally higher frequency of the p.P34S variant in our sporadic ALS cohort compared to the ExAC server is not significant ($P = 0.29$; Fisher's exact test). Moreover, also in the ExAC data set a substantial number of p.P34S carriers are excluded from the allele count due to low genotype quality, and their frequency may thus be considerably higher. Pooling of all sporadic ALS patients with a p.P34S variant from previous studies (Chaussenot *et al.*, 2014; Chio *et al.*, 2015; Ronchi *et al.*, 2015) and this work (in total 10 out of 876; 1.1%) and comparison with the respective pooled ethnic and geographically matched controls (6/1044 p.P34S variant carriers; 0.6%) also does not reveal a significant difference between cases and controls ($P = 0.21$; Fisher's exact test; for overview see Table 1).

The four patients with the p.P34S variant identified in this study, two males and two females, had spinal onset disease. Age of onset was 61, 61, 68, and 70 years and survival times were 27, 29, 31 and 38 months, respectively. The median survival time was thus 30 months, which is in agreement with the median survival of 29 months observed in the total sporadic ALS cohort that was tested (survival time available from 303 out of 355 patients).

Taken together, we performed a screen for *CHCHD10* variants in the largest ALS cohort to date. Our data suggest that *CHCHD10* mutations are rare in ALS patients without a familial background of the disease. We failed to detect an enrichment of the previously reported p.P34S variant in patients. Moreover, the disease course of p.P34S variant carriers was not different from the overall cohort that was tested. This contrasts with the conspicuously slow disease progression observed in most patients with motor neuron disease carrying a *CHCHD10* mutation for which pathogenicity is supported by co-segregation data (Bannwarth *et al.*, 2014; Johnson *et al.*, 2014; Müller

Table 1 Overview of *CHCHD10* p.P34S variant distribution in sporadic ALS cases and controls

Total cases	Number of cases with p.P34S variant	% cases with p.P34S variant	Total control	Number of controls with p.P34S variant	% controls with p.P34S variant	*P*-value (difference in frequency in cases versus controls, where applicable; Fisher's exact test)	Reference
80	2	2.5	200	0	0	0.08	Chaussenot *et al.*, 2014
217	1	0.5	286	0	0	0.43	Ronchi *et al.*, 2015
224	3	1.3	165	0	0	0.27	Chiò *et al.*, 2015
355	4	1.1	393	6	1.5	0.76	This work
n.a.	n.a.	n.a.	1499	9	0.6	n.a.	ExAC data set
876	**10**	**1.1**	**2543**	**15**	**0.6**	**0.11**	**Total**

et al., 2014; Kurzwelly *et al*., 2015; Penttila *et al*., 2015). Overall, we therefore concur with the most recent letter by Dobston-Stone *et al*. (2015) who questioned the pathogenicity of the *CHCHD10* p.P34S variant. Its absence in the local controls that were part of the first *CHCHD10* studies in sporadic ALS may be due to the relatively small control cohorts ($n = 200$, 286 and 165, respectively) and/or regional inhomogeneity of the p.P34S distribution. Of note, the ExAC sequence data set was not publicly available when the p.P34S variant was first described and estimated to be pathogenic, partially based on its absence in smaller reference databases (Chaussenot *et al*., 2014). Moreover, considering the non-significant slight trend towards a more frequent detection of p.P34S in sporadic ALS cohorts when taking together all *CHCHD10* mutational screenings in sporadic ALS and the ExAC data set (Table 1), it cannot be fully excluded that screening of even larger cohorts will reveal p.P34S as a weak risk factor for ALS/FTD.

Judgement of the p.P34S variant in *CHCHD10* is relevant for genetic counselling, especially in view of the fact that it is observed at a frequency of up to 1.5% (this report) in control individuals of European or Australian origin (Table 1). The case of p.P34S in *CHCHD10* generally highlights the importance of well-matched, sufficiently large control cohorts when assessing the pathogenicity of specific genetic variants in the absence of co-segregation data. It also recommends a critical re-assessment of the pathogenicity of published disease-related variants in view of the increasing number of exome/genome control sequence data that are becoming publicly available.

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