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CHCHD10 variants in Amyotrophic Lateral Sclerosis: where is the evidence?

Project MinE ALS Sequencing Consortium*

Abstract

Objective—After the initial report of a *CHCHD10* mutation in mitochondrial disease with features resembling amyotrophic lateral sclerosis (ALS), *CHCHD10* mutations have been considered to be a frequent cause for ALS. However, the exact pathogenicity and clinical significance of these mutations remain unclear. Here, we aimed to determine the role of *CHCHD10* mutations in ALS.

Methods—We analyzed 4,365 whole-genome sequenced ALS patients and 1,832 controls from 7 different countries and examined all non-synonymous single nucleotide variants (SNVs) in *CHCHD10*. These were tested for association with ALS, independently and in aggregate using several genetic burden tests (including SKAT, SKAT-O and Firth logistic regression).

Results—We identified three new variants in cases, but only one was ALS-specific. Also, one control-specific mutation was identified. There was no increased burden of rare coding mutations among ALS patients compared to controls ($P = 0.86$, $P = 0.86$ and $P = 0.88$ for SKAT, SKAT-O and Firth, respectively). The few carriers with potential pathogenic *CHCHD10* mutations exhibited a slowly progressive ALS-like phenotype with atypical features such as myopathy and deafness.

Interpretation—*CHCHD10* mutations seem to be a far less prevalent cause of pure ALS than previously suggested, and instead appear related to more complex phenotypes. There appears to be insufficient evidence for the pathogenicity of most previously reported variants in pure ALS. This study shows that routine testing for *CHCHD10* mutations in pure ALS is not recommended and illustrates the importance of sufficient genetic and functional evidence in establishing pathogenicity of genetic variants.

Corresponding author: Jan H. Veldink, Department of Neurology and Neurosurgery, University Medical Centre Utrecht, Department of Neurology G03.228, P.O. Box 85500, 3508 GA Utrecht, The Netherlands, J.H.Veldink@umcutrecht.nl.

*Members and affiliations of the Project MinE ALS Sequencing Consortium are listed in Supplementary Information.

Author Contributions

GT, WR and JV contributed to the conception and design of the study; GT, WR, SP, RS, AD, MM, RM, WS and KK contributed to the acquisition and analysis of data; GT, WR, AA, KM, WRo, PS, CS, ME, AB, MP, JM, JG, PD, OH, JL, LB and JV contributed to drafting the text and preparing the figures. A full list of Project MinE ALS Sequencing Consortium author contributions and affiliations are listed in Supplementary Table 2.

Potential Conflicts of Interest

Nothing to report

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurological disease characterized by the degeneration of both upper and lower motor neurons, leading to progressive muscle weakness and respiratory failure.[1] Using next-generation sequencing, mutations in several genes have been reported, especially in the minority of cases with a positive family history of ALS.[2] These discoveries have not only led to increased understanding of the pathophysiology of ALS and the possible development of specific therapeutic agents, but also play an important role in genetic counselling.

CHCHD10 was proposed as a new candidate gene for ALS, after a novel p.Ser59Leu mutation in *CHCHD10* was described as co-segregating with a complex variable phenotype, including cognitive decline resembling frontotemporal dementia (FTD), cerebellar ataxia, myopathy, sensorineural deafness and an ALS-like motor neuron disease.[3] Although subsequent screening in different populations has led to the description of over 20 mutations in *CHCHD10* (most of which are located in exon 2)[4–7] in ALS and other neurodegenerative diseases, our certainty in the causality of these variants for ALS remains an open question.[8, 9]

Typically, to establish the causality of the identified *CHCHD10* variants, investigators used predictive software for individual mutations to indicate a deleterious effect and (virtual) absence in public databases. However, it is widely accepted that these criteria alone are insufficient proof of causality for low-frequency variants[8], especially if those variants were identified only once in a single index case. Consequently, these lenient criteria for claiming causality between a variant and disease might lead to false positive reports due to inadequate coverage in exome-captured data, geographically-specific genetic variation and underpowered studies.[10, 11] Nevertheless, influential online resources and literature for genetic counseling such as Clinvar (https://www.ncbi.nlm.nih.gov/clinvar) and the Amyotrophic Lateral Sclerosis Online genetics Database (ALSoD, http://alsod.iop.kcl.ac.uk/) have already adopted *CHCHD10* variation as causal for ALS and sources such as GeneReviews suggest genetic testing in the clinic if the phenotype is unusual and other more common genes of ALS have been excluded.[12]

To determine the veracity of claims that *CHCHD10* variants are causal in ALS and valid to use in the clinic, we have set out to investigate the genetic contribution of *CHCHD10* variants in a large international cohort of whole-genome sequenced ALS patients and controls.

Materials and Methods

Sample collection

DNA was isolated from whole blood samples collected from 4,853 ALS patients from 7 different populations (Belgium, Ireland, The Netherlands, The United Kingdom, The United States of America, Spain and Turkey) and 1,991 controls matched for age, geographical location and sex. All patients and control subjects provided written informed consent and the relevant institutional review boards approved this study.

Sequencing and analysis

DNA samples were sequenced using PCR-free library preparation and paired-end sequencing on the HiSeq 2000 (100 bp) and HiSeq X platform (150 bp) (Illumina®, San Diego, USA). Reads were aligned to the hg19 human genome build using the Isaac alignment software and the Isaac variant caller was used to call and filter single nucleotide variants using standard quality control (QC) parameters.[13] Additional QC removed duplicated or poorly called individuals (genotype missingness > 5%, Ti/Tv > 2.092, het/hom ratio > 3.1) and genomic sites (high or low depth of coverage, aggregated passing rate < 0.7 across the sample, missingness > 5%, HWE $p < 1 \times 10^{-6}$). We also removed all closely related (kinship coefficient > 0.0625) and sex-check failing samples based on comparison of phenotype and sequencing data.[14] The genomic region of *CHCHD10* (NCBI Reference Sequence: NG_034223.1) was isolated from the VCFs and variants were annotated using Variant Effect Predicitor.[15]

Burden Testing

Gene regions were isolated based on their canonical transcripts in the Ensembl database (http://www.ensembl.org). Within these regions, single nucleotide variants (SNVs) that were annotated as missense or loss-of-function mutations with a minor allele frequency (MAF) <1% in the control population and public databases were selected for burden testing. Burden testing on cases and controls was performed using bidirectional sequence kernel association test (SKAT) together with SKAT-O to account for an unidirectional effect (which is more likely in the case of mainly damaging variants) and Firth corrected logistic regression.[16–18] Association tests were corrected for population stratification using the first 10 principal components. Additionally, 100.000 permutations were performed with SKAT-O and Firth logistic regression to obtain the empirical p-values. Statistical analyses were carried out using R software (http://www.r-project.org).

Results

To investigate variants in *CHCHD10*, we analyzed all rare, non-synonymous SNVs in the whole-genome sequencing data of 4,365 ALS (± FTD) samples together with 1,832 unaffected controls. We identified seven SNVs in ALS cases, three of which were not previously reported (Table 1). Screening of controls revealed that only three out of these seven variants were ALS-specific, as the other four variants were also found in controls. Additionally, one control-specific SNV was identified.

No increased burden of rare variants

None of the different association tests showed a significant increased burden of rare non-synonymous variants in *CHCHD10* among ALS patients (SKAT: P = 0.86; SKAT-O: $P = 0.86$ and Firth: $P = 0.88$; Table 2). As a positive control, we tested three other genes (*SOD1*, *FUS* and *TARDBP*), which are known to harbor rare pathogenic SNVs in ALS.[19] These genes did yield significant association statistics in both SKAT-O as well as Firth, indicating a unilateral effect (Table 2).

Additional clinical information on carriers

Only three rare missense mutations in *CHCHD10* were specific to ALS cases (Supplementary Table 1). The previously unreported p.Arg11Gly mutation was identified in a single female ALS case from the United States without cognitive involvement and a negative family history for ALS or dementia. We identified three cases with the previously reported p.Arg15Leu variant: one Dutch and two American cases, one of which was already included in the previous study by Johnson et al. (ND11809).[5] Although both American cases had a positive family history, the additional Dutch ALS patient did not have a family history of ALS or dementia. Similar to previously described carriers, the clinical phenotype in this patient was characterized by very slow progression with both upper and lower motor neuron involvement, a long diagnostic delay of two years and a disease duration of over eight years after onset.[5, 6, 20] Interestingly, besides motor neuron disease, this patient presented with an atypical phenotype including deafness, weakness of the proximal upper extremities and reduced tendon reflexes. Unfortunately, no muscle biopsies were performed to detect myopathy. The third case-specific mutation (p.Pro80Leu), previously reported in an Italian ALS patient with an abnormal muscle biopsy (COX deficiency), was found in a Belgian ALS patient.[7] This patient also presented with an atypical myopathy-like clinical phenotype with proximal lower limb weakness and high serum creatine kinase levels (up to 1800 U/l). The clinical features at the time of presentation prompted the neurologist to request a muscle biopsy, which showed neurogenic atrophy, but without histochemical analysis for COX.

Discussion

CHCHD10 was proposed as a new candidate gene for ALS following the initial report of a p.Ser59Leu variant, which was detected in a family with a complex phenotype including ataxia, myopathy, dementia and a progressive motor neuron disease resembling ALS.[3] Subsequently, several studies screened for *CHCHD10* mutations in ALS patients and healthy controls and claimed pathogenicity for multiple rare missense variants.[4–6] In this study, we used whole-genome sequencing data on a large international cohort of ALS patients to investigate the frequency of *CHCHD10* variants and evaluated the genetic evidence for their pathogenicity.

In our cohort of 4,365 ALS patients and 1,832 controls, we only detected three rare, case-specific, missense variants, two of which have been previously reported. The only remaining novel ALS-specific variant, a heterozygous c.31C>G variant resulting in a p.Arg11Gly amino acid change, was found in a single ALS case and is therefore of unknown significance. Furthermore, we also identified a rare missense variant (p.Ala72Val) in a single control sample, indicating that unique coding variants can be found in controls as well. Together with our data, there are now 13 reported rare nonsynonymous variants in *CHCHD10* in cases diagnosed with pure ALS, most of which are concentrated in exon 2 (Fig 1). Missense mutations in exon 2 were also detected in other neurodegenerative diseases, some of which closely related to ALS. Although this might hint towards pleiotropy, it is important to realize that most reported variants were unique to a single case or family and that this exon is only moderately covered in whole-exome sequencing-based public databases such as ExAC, making it prone to false positive reports. For instance, at the

p.Arg15Leu variant site, chr22:24109778, the fraction of individuals in ExAC with coverage of 20x or higher was only 0.0003.[10]

In order to interpret the collection of rare variants in cases and/or controls, we tested whether there is an increased burden of rare non-synonymous variants in *CHCHD10* among ALS patients. The results of the association tests show no significant association between rare coding variants in *CHCHD10* and ALS, whereas rare variants in *FUS, TARDBP* and *SOD1* did show a significant association of non-synonymous variants in ALS using both SKAT-O and Firth corrected association tests. SKAT p-values were not significant, which was expected as variants in these genes are known to be damaging, not protective. Although mutations in these genes are considered rare but not uncommon in sporadic ALS, the difference in association signal does not exclude pathogenicity of *CHCHD10* variants in ALS; it does however indicate a very low prevalence.

In the absence of linkage or a statistically significant burden test, all variants that are solely observed in a single index case do not meet criteria for pathogenicity.[8] Only variants that occur in multiple unrelated cases (and absent or extremely rare in controls) are potentially more interesting. Together with previous reports, only six *CHCHD10* variants have met this criterion (Table 3). Some of these variants are already listed as (possibly) pathogenic in public databases such as ClinVar despite the fact that other criteria for establishing pathogenicity were often not investigated.

So far, the most convincing evidence for *CHCHD10* pathogenicity was provided for the p.Ser59Leu variant, using both clinical and genetic data on multiple affected and unaffected family members. The clinical phenotype described in these carriers, however, is not pure ALS and includes atypical features such as deafness, myopathy, cerebellar ataxia and Parkinsonism.[3] With our focus on typical ALS, we will critically appraise the genetic evidence for the five other reported variants.

Similar to previous observations, the most frequent rare non-synonymous SNV in our dataset was the heterozygous p.Pro34Ser, which was present in 37 cases (0.85%) as well as 15 control samples (0.82%) (corrected $\chi^2(1) = 0.00$ $P = 0.98$). Despite initial reports of possible pathogenicity of this variant in pure ALS ± FTD, our data adds to the increasing evidence that the p.Pro34Ser mutation in *CHCHD10* is probably not pathogenic.[21–24] Recent *in vitro* studies still support p.Pro34Ser pathogenicity as similar cellular pathology between $CHCHD10^{S59L}$ and $CHCHD10^{P34S}$ mutant cell lines was shown.[25] Despite the *in vitro* findings, the fact that the p.Pro34Ser variant is as common in ALS patients as in the general population, indicates that an apparently abnormal phenotype in transfected cell lines alone does not justify classifying the p.Pro34Ser variant as an ALS causing mutation and indicates the substantial limitation of these models to represent human ALS-pathology.

Previous screening of a subset of sporadic ALS patients with COX-deficient muscle biopsies led to the discovery of a c.244C>T substitution (p.Pro80Leu) in exon 2, which was subsequently reported in two sporadic and one familial ALS cases in Italy and Canada.[7, 26] We have identified an additional sporadic case in our Belgian cohort with a similar atypical clinical phenotype. However, the allele frequency of this variant in ALS cases after

exclusion of possibly overlapping cohorts (5/12700 = 0.0004) is almost identical to the general population in the ExAC database (32/92470 = 0.0003, corrected $\chi^2(1) = 0.00$ $P = 0.99$). Moreover, the frequency in the ExAC database might even be an underestimation as exon 2 is only moderately represented (Figure 1).

The fourth and fifth variants which were identified in multiple ALS cases are the p.Pro96Thr and p.Tyr135His mutations. These variants are located in exon 3 and, similar to p.Pro80Leu, pathogenicity is unlikely due to similar allele frequencies in control samples.[22, 27–29] Notably, the p.Pro96Thr is the only variant which was found to be homozygous in 3 out of 5 cases. Given its high frequency in the African population in ExAC (692/2704 = 0.2559) however, a pathogenic recessive nature of this mutation seems highly unlikely.

The last variant, c.44G>T (p.Arg15Leu), was previously detected in six families with ALS and one sporadic ALS case.[5, 6, 20, 30] This variant is probably of the greatest interest in ALS as it was identified in multiple cohorts, segregated with disease in familial cases (although there were three unaffected carriers in one family, reflecting incomplete penetrance) and was absent in with in any of the screened controls.[6] Here, we report two new carriers: one in the Dutch cohort and one in the US cohort (the other US carrier has already been reported). Although limited, the available clinical data for these patients is similar to previously reported carriers (predominant lower-motor neuron signs and slow disease progression) with some atypical features in one patient (bilateral hearing loss and proximal onset), supporting an ALS-like clinical phenotype.[6, 20] However, the percentage of ALS cases associated with this variant is 0.1% (9/6,797 non-overlapping cases) making it a possibly pathogenic but very rare *CHCHD10* variant for motor-neuronopathy.

The association of *CHCHD10* mutations in motor-neuron disorder resembling ALS is further illustrated by the c.197G>T (p.Gly66Val) variant, which was originally described in a Finnish familial ALS patient with slowly ascending progressive motor neuronopathy. This variant was later shown to cause a lower motor neuron phenotype without upper-motor neuron or cognitive involvement as it was identified in 75 Finnish carriers with hereditary, late onset spinal motor neuronopathy (SMAJ), Charcot-Marie Tooth disease Type 2 or both.[6, 31–33]

Overall, there is evidence that some variants in *CHCHD10* are associated with motor neuron degeneration, particularly in combination with clinical features that suggest mitochondrial dysfunction, such as myopathy or hearing-loss. In the case of pure ALS however, our results indicate that most rare genetic variants in *CHCHD10* are detected in both cases and controls at similar frequencies. Thus, we find little evidence that *CHCHD10* variants are a prevalent cause of pure ALS as has previously been suggested and do not support routine diagnostic or predictive testing for *CHCHD10* variants in pure ALS.[5] Our study underlines the importance of gaining robust genetic and functional evidence to establish pathogenicity before advocating gene testing in a clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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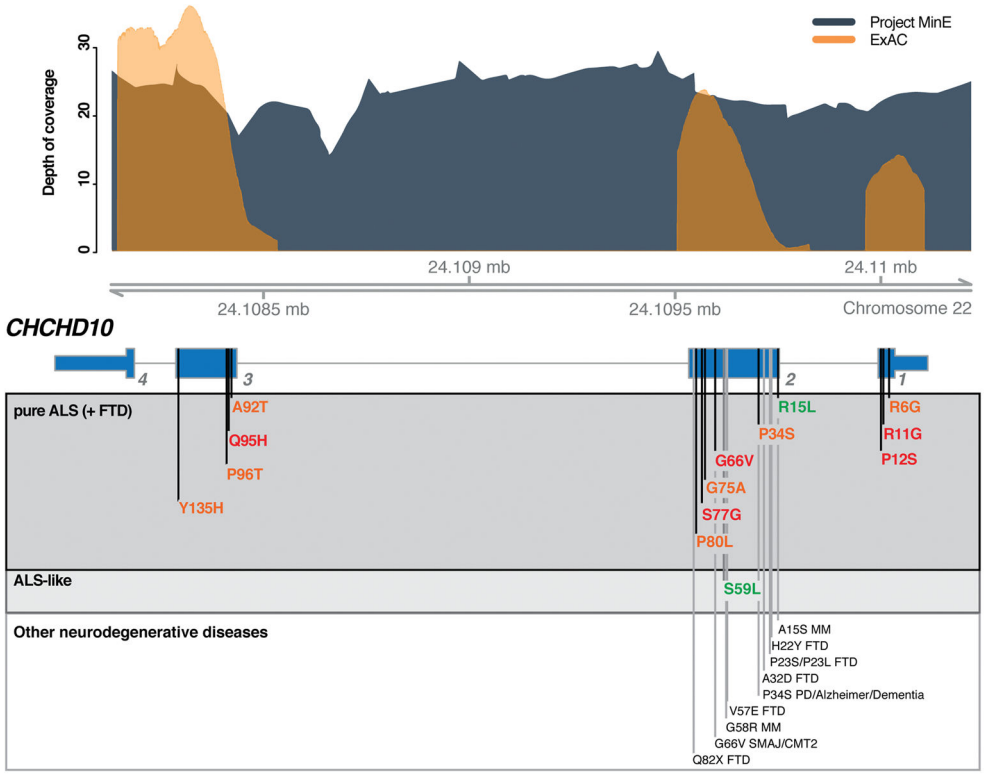

Figure 1. Non-synonymous *CHCHD10* variants in neurodegenerative diseases

Overview of rare non-synonymous variants in ALS and other neurodegenerative diseases and their exonic location in *CHCHD10*. The top panel shows depth of coverage of *CHCHD10* in the ExAC public database (orange) and Project Mine whole-genome sequencing data (blue-grey) (http://databrowser.projectmine.com). The grey panel shows all variants reported in pure ALS ± FTD; variants in green were present in multiple seemingly unrelated cases and absent in controls, orange variants were identified in both cases as well as controls and red variants were found in a single ALS case. The light grey panel shows variants reported in a more extensive phenotype that includes motor neuron disease. The bottom panel shows all variants and their location that were reported in other neurodegenerative diseases (MM = mitochondrial myopathy, PD = Parkinson's disease, SMAJ = late onset spinal motor neuronopathy, CMT2 = Charcot-Marie Tooth Type 2).

Table 1

***CHCHD10* Variants in Project Mine**

Overview of rare (MAF <1%) single nucleotide variants, functionally annotated as missense of loss of function in a total of 4,365 ALS and 1,832 control samples with the genomic location, location in transcript NM_213720.1 and predicted amino acid change, allele counts and corresponding minor allele frequencies (MAF) together with the MAF of the European population in the ExAC database. If the variant was not identified the allele frequency was expected to be below the minimal MAF of individuals with a site coverage of 20x or higher.

Genome	Transcript	Consequence	Alleles Cases	Alleles Controls	MAF Cases	MAF Controls	MAF ExAC
22:24108321 A>G	c.403T>C	p.Tyr135His	3	1	0.00034	0.00027	0.00030
22:24109583 G>A	c.239C>T	p.Pro80Leu	1	0	0.00011	< 0.0027	0.00047
22:24109598 C>G	c.234G>C	p.Gly75Ala	1	1	0.00011	0.00027	0.00002
22:24109607 G>A	c.225C>T	p.Ala72Val	0	1	< 0.00011	0.00027	0.00005
22:24109722 G>A	c.100C>T	p.Pro34Ser	37	15	0.00423	0.00409	0.00298
22:24109778 C>A	c.44G>T	p.Arg15Leu	3	0	0.00034	< 0.00027	< 0.02745
22:24110031 G>C	c.31C>G	p.Arg11Gly	1	0	0.00011	< 0.00027	< 0.00005
22:24110046 G>C	c.16C>G	p.Arg6Gly	1	2	0.00011	0.00055	0.00007

Table 2

Burden Testing

Results of burden test analysis using SKAT, SKAT-O and Firth corrected association testing on rare (MAF<1%) non-synonymous single nucleotide variants in *CHCHD10* and known ALS genes. Nvar indicates the number of SNVs which were taken into account for association testing.

Gene	nvar	SKAT p-value	SKAT-O p-value	permuted SKAT-O	Firth Test p-value	permuted Firth Test
CHCHD10	8	0.8606	0.8599	0.8792	0.7248	0.7382
SOD1	27	0.3595	0.0008	0.0012	0.0001	0.0001
FUS	22	0.6168	0.0224	0.0215	0.0095	0.0086
TARDBP	19	0.6446	0.0340	0.0371	0.0192	0.0207

Table 3

Non-synonymous *CHCHD10* variants in multiple ALS / FTD cases

Overview of total number of alleles and variant alleles, evidence of segregation in pedigrees and reported clinical significance in ClinVar database of variants that were previously and currently reported in multiple (>1) seemingly unrelated ALS or FTD patients. Alleles that were present in affected or unaffected family members were excluded. No overlap indicates the minimum number of alleles that were screened in non-overlapping cohorts (after removal of UK, US and SP cohorts).

	Project Mine		**Previous reports**			
Variants:	***ALS***	***Controls***	***ALS***	***Controls***	***Segregation in Pedigree(s):***	***ClinVar***
p.Arg15Leu	3	0	7	0	Yes	Conflicting
p.Pro34Ser	37	15	20	25	No *	Pathogenic
p.Ser59Leu	0	0	2	0	Yes	Pathogenic
p.Pro80Leu	1	0	4	0	Unknown	Pathogenic
p.Pro96Thr **	2	2	6	3	Unknown	Unknown
p.Tyr135His	3	1	1	0	Unknown	Unknown
Total screened	8730	3664	7560	6604		
No overlap	**5140**	**2778**				

* In a pedigree with FTD[24]

** Allele counts were not provided in all reports[27]