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

Project MinE ALS Sequencing Consortium

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

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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. The exact pathogenicity and clinical significance, however, of these mutations remain unclear. Here, we aimed to determine the role of *CHCHD10* mutations in ALS.

Methods: We included 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 and SKAT-O).

Results: We identified three new variants in cases, but only one was case-specific. Also, one control-specific mutation was identified. There was no increased burden of rare coding mutations among ALS patients compared to controls ($P = 0.88$ and $P = 1.00$ for SKAT and SKAT-O, 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, but 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.

Abbreviations

Amyotrophic lateral sclerosis (ALS); Frontotemperal dementia (FTD); minor allele frequency (MAF); Sequence kernel association test (SKAT); single nucleotide variant (SNV);

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 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* in ALS and other neurodegenerative diseases (most of which are located in exon 2)[4-7], 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 functional effect predictors for individual mutations 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 in single families but never followed up in additional samples. Consequently, these lenient criteria for claiming causality between a variant and disease might lead to false positive reports due to a combination of factors: incomplete penetrance, lack of adequate coverage in exome-captured data, rare genetic variation that may be geographically specific, or simply no relationship between the variant and disease (discoverable through investigation of increased sample sizes).[10, 11] Nevertheless, influential online resources and literature for genetic counseling have already adopted *CHCHD10* variation as causal for ALS and suggest genetic testing in the clinic (http://neuromuscular.wustl.edu/).[12]

To determine the clinical impact and importance of genetic testing for claimed *CHCHD10* variants in ALS, we have set out to investigate the true 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, more likely in the case of mainly damaging variants.[16] Association tests were corrected for population stratification using the first 10 principal components. Additionally, 100.000 permutations were performed with SKAT-O to obtain the empirical SKAT-O p-value. 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 case-specific, as the other four variants were also found in controls. Additionally, one control-specific SNV was identified.

No increased burden of rare variants

Neither the SKAT nor the SKAT-O association tests showed a significant increased burden of rare non-synonymous variants in *CHCHD10* among ALS patients (P = 0.88 and P = 1.00, respectively; 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.[17] These genes did yield a significant SKAT-O association statistic *(*P = 0.008, P = 0.02, and P = 0.03 respectively; Table 2).

Additional clinical information on carriers

Only three rare missense mutations in *CHCHD10* were specific to ALS cases (Table 2). 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, 18] Interestingly, besides motor neuron disease, this patient presented with an atypical phenotype including deafness, weakness of the proximal upper extremities and low tendon reflexes. Unfortunately, no muscle biopsies were performed. 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 to be 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

189 rare missense variants.[4-6] In this study, we used whole-genome sequencing data on
190 a large international cohort of ALS patients to investigate the frequency of *CHCHD10*
191 variants and evaluated the genetic evidence for their pathogenicity.

192 In our cohort of 4,365 ALS patients and 1,832 controls, we only detected three
193 rare, case-specific, missense variants, two of which have been previously reported.
194 The only remaining novel ALS-specific variant, a heterozygous c.31C>G variant
195 resulting in a p.Arg11Gly amino acid change, was found in a single ALS case and is
196 therefore of unknown significance. Furthermore, we also identified a rare missense
197 variant (p.Ala72Val) in a single control sample, indicating that unique coding variants
198 can be found in controls as well. Together with our data, there are now 13 reported
199 rare nonsynonymous variants in *CHCHD10* in pure ALS, most of which are
200 concentrated in exon 2 (Figure 1). Missense mutations in exon 2 were also detected
201 in other neurodegenerative diseases, some of which closely related to ALS. Although
202 this might hint towards pleiotropy, it is important to realize that most reported variants
203 were unique to a single case or family and that this exon is only moderately covered
204 in whole-exome sequencing-based public databases such as ExAC, making it prone
205 to false positive reports.[10]

206 In order to interpret the collection of rare variants in cases and/or controls, we
207 tested whether there is an increased burden of rare non-synonymous variants in
208 *CHCHD10* among ALS patients. The results of the SKAT and SKAT-O association
209 tests show no significant association between rare coding variants in *CHCHD10* and
210 ALS, whereas genes which are known for causative rare variants in ALS did show a
211 significant association of non-synonymous variants in ALS in SKAT-O only (which
212 was expected as variants in these genes are known to be damaging, not protective).

In the absence of linkage or a statistically significant burden test, all variants that are solely observed in a single case do not meet criteria for pathogenicity.[8] Only variants that occur in multiple unrelated cases (and no controls) are potentially more interesting. Together with previous reports, only six *CHCHD10* variants have met this criterion (Table 4). Some of these variants are already listed as pathogenic in public databases such as ClinVar (https://www.ncbi.nlm.nih.gov/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 $\pm$ FTD, our data adds to the increasing evidence that the p.Pro34Ser mutation in *CHCHD10* is probably not pathogenic.[19-22] Recent *in vitro* studies still support p.Pro34Ser pathogenicity as similar cellular pathology between $CHCHD10^{S59L}$ and $CHCHD10^{P34S}$ mutant cell lines was shown.[23] 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 a HeLa cellular phenotype alone does not justify classifying

236 the p.Pro34Ser variant as an ALS causing mutation and could merely indicate the
237 limitation of these models to represent human ALS-pathology.

238 Previous screening of a subset of sporadic ALS patients with COX-deficient
239 muscle biopsies led to the discovery of a c.244C>T substitution (p.Pro80Leu) in exon
240 2, which was subsequently reported in two sporadic and one familial ALS cases in
241 Italy and Canada.[7, 24] We have identified an additional sporadic case in our Belgian
242 cohort with a similar atypical phenotype. However, the allele frequency of this variant
243 in ALS cases after exclusion of possibly overlapping cohorts (5/12700 = 0.0004) is
244 almost identical to the general population in the ExAC database (32/92470 = 0.0003,
245 corrected $\chi^2(1) = 0.00$ $P = 0.99$). Moreover, the frequency in the ExAC database
246 might even be an underestimation as exon 2 is only moderately represented (Figure
247 1).

248 The fourth and fifth variants which were identified in multiple ALS cases are
249 the p.Pro96Thr and p.Tyr135His mutations. These variants are located in exon 3
250 and, similar to p.Pro80Leu, pathogenicity is unlikely due to similar allele frequencies
251 in control samples.[20, 25-27] Notably, the p.Pro96Thr is the only variant which was
252 found to be homozygous (in 3 out of 5 cases). Given its high frequency in the African
253 population in ExAC (692/2704 = 0.2559) however, a pathogenic recessive nature of
254 this mutation seems highly unlikely.

255 The last variant, c.44G>T (p.Arg15Leu), was previously detected in six
256 families with ALS and one sporadic ALS case.[5, 6, 18, 28] This variant is probably of the
257 most interest in ALS as it was identified in multiple cohorts, with absence in any of
258 the screened controls, and segregates with disease in familial cases (although there
259 were three unaffected carriers in one of the families, possibly due to incomplete

penetrance).[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 seems to be similar to that reported in other carriers (predominant lower-motor neuron symptoms and slow disease progression) with some atypical symptoms in one patient (bilateral hearing loss and proximal onset).[6, 18] This again points towards a distinct ALS-like clinical phenotype. However, the percentage of ALS cases which might be explained due to this variant is 0.1% (9/6,797 non-overlapping cases) making it a possibly pathogenic but very rare *CHCHD10* variant in motor-neuron disease.

The association of *CHCHD10* mutations in motor-neuron disease 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 neuron disease. 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 neuropathy (SMAJ), Charcot-Marie Tooth disease Type 2 or both.[6, 29-31]

Overall, there seems to be potential evidence for the involvement of *CHCHD10* in neurodegenerative diseases, 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 rare genetic variants in *CHCHD10* can be detected in both cases and controls at similar frequencies. Therefore, *CHCHD10* variants seem to be a far less prevalent cause of pure ALS than previously suggested.[5] This study shows that routine testing for *CHCHD10* variants in pure ALS is not recommended and illustrates the importance

of sufficient genetic and functional evidence in establishing pathogenicity of genetic variants.

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Conflicts of Interest

Nothing to report.

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	-	0.00047
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	-	0.00027	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	-	-
22:24110031 G>C	c.31C>G	p.Arg11Gly	1	0	0.00011	-	-
22:24110046 G>C	c.16C>G	p.Arg6Gly	1	2	0.00011	0.00055	0.00007

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.

Gene	nvar	SKAT p-value	SKAT-O p-value	permuted SKAT-O
CHCHD10	8	0.8773	1	1
SOD1	27	0.3063	0.0006	0.0008
FUS	22	0.6156	0.0245	0.0242
TARDBP	19	0.6167	0.0283	0.0300

Table 2. Burden Testing Results of burden test analysis using SKAT and SKAT-O 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.

Mutation	Cohort	Sex	AAO (yr)	Surv (mo)	SOO	Myopathy	Muscle Biopsy	Deafness	Ataxia	Sensory deficits	FTD	Family History
p.Pro80Leu	BE	M	61	54	LE	Yes	Yes	No	No	No	No	No
p.Arg15Leu	NL	M	73	76	UE	Possible	No	Yes	No	No	No	No
p.Arg15Leu	US	F	42	-	LE	-	-	-	No	No	No	ALS
p.Arg15Leu	US	F	71	-	LE	-	-	-	No	No	No	ALS
p.Arg11Gly	US	F	47	64	LE	-	-	-	No	No	No	Myasthenia

Table 3. Clinical information on carriers of observed ALS specific missense variants. Overview of known clinical data available for carriers of ALS-specific rare *CHCHD10* missense mutations in our study; Country of sample origin (Cohort)(BE = Belgium, NL = The Netherlands, US = The United States of America), Age of onset in years (AAO), Survival after onset in months (Surv) Site of onset (SOO) (LE = lower extremities, UE = upper extremities), Clinical indications for Myopathy (Myopathy).

	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[22]
**Allele counts were not provided in all reports[25]

Table 4. 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).

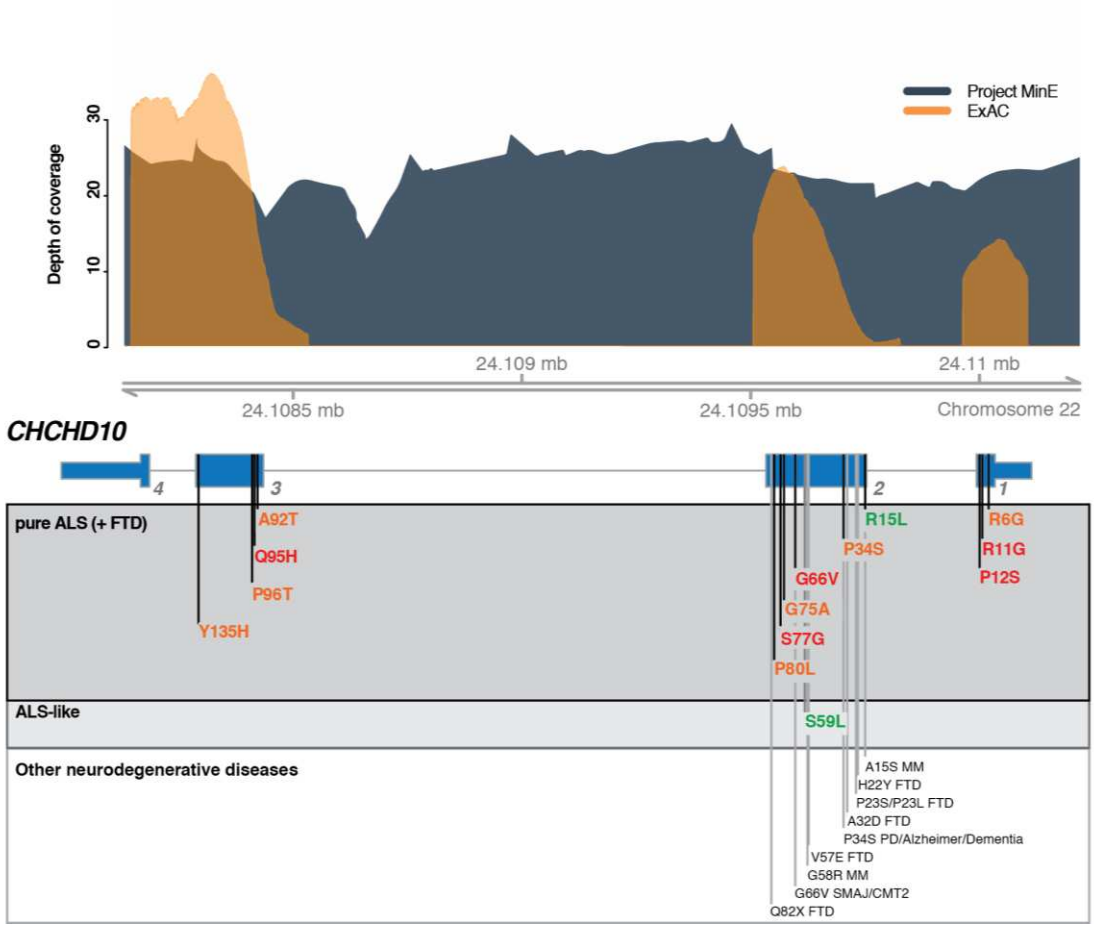

Figure 1. Non-synonymous *CHCHD10* variants in neurodegenerative diseases. Overview of rare non-synonymous variants in ALS and other neurodegenerative diseases and their exonic location in *CHCHD10*. The top panel shows depth of coverage of *CHCHD10* in the ExAC public database (orange) and Project Mine 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

389 (MM = mitochondrial myopathy, PD = Parkinson's disease, SMAJ = late onset spinal

390 motor neuronopathy, CMT2 = Charcot-Marie Tooth Type 2).

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