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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.

**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

## 46 **Abstract**

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

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

57 **Results:** We identified three new variants in cases, but only one was case-specific.  
58 Also, one control-specific mutation was identified. There was no increased burden of  
59 rare coding mutations among ALS patients compared to controls ( $P = 0.88$  and  $P =$   
60  $1.00$  for SKAT and SKAT-O, respectively). The few carriers with potential  
61 pathogenic CHCHD10 mutations exhibited a slowly progressive ALS-like phenotype  
62 with atypical features such as myopathy and deafness.

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

## 70 **Abbreviations**

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

74

## 75 **Introduction**

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

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

92 Typically, to establish the causality of the identified CHCHD10 variants,  
93 investigators used functional effect predictors for individual mutations and (virtual)  
94 absence in public databases. However, it is widely accepted that these criteria alone

95 are insufficient proof of causality for low frequency variants<sup>8</sup>, especially if those  
96 variants were identified in single families but never followed up in additional samples.  
97 Consequently, these lenient criteria for claiming causality between a variant and  
98 disease might lead to false positive reports due to a combination of factors:  
99 incomplete penetrance, lack of adequate coverage in exome-captured data, rare  
100 genetic variation that may be geographically specific, or simply no relationship  
101 between the variant and disease (discoverable through investigation of increased  
102 sample sizes).<sup>10, 11</sup> Nevertheless, influential online resources and literature for  
103 genetic counseling have already adopted CHCHD10 variation as causal for ALS and  
104 suggest genetic testing in the clinic (<http://neuromuscular.wustl.edu/>).<sup>12</sup>

105         To determine the clinical impact and importance of genetic testing for claimed  
106 CHCHD10 variants in ALS, we have set out to investigate the true genetic  
107 contribution of CHCHD10 variants in a large international cohort of whole genome  
108 sequenced ALS patients and controls.

109

## 110 **Materials and Methods**

### 111 **Sample collection**

112 DNA was isolated from whole blood samples collected from 4,853 ALS patients from  
113 7 different populations (Belgium, Ireland, The Netherlands, The United Kingdom, The  
114 United States of America, Spain and Turkey) and 1,991 controls matched for age,  
115 geographical location and sex. All patients and control subjects provided written  
116 informed consent and the relevant institutional review boards approved this study.

### 117 **Sequencing and analysis**

118 DNA samples were sequenced using PCR-free library preparation and paired-end  
119 sequencing on the HiSeq 2000 (100 bp) and HiSeq X platform (150 bp) (Illumina®,  
120 San Diego, USA). Reads were aligned to the hg19 human genome build using the  
121 Isaac alignment software and the Isaac variant caller was used to call and filter  
122 single nucleotide variants using standard quality control (QC) parameters.<sup>13</sup>  
123 Additional QC removed duplicated or poorly called individuals (genotype  
124 missingness > 5%, Ti/Tv > 2.092, het/hom ratio > 3.1) and genomic sites (high or low  
125 depth of coverage, aggregated passing rate < 0.7 across the sample, missingness >  
126 5%, HWE  $p < 1 \times 10^{-6}$ ). We also removed all closely related (kinship coefficient >  
127 0.0625) and sex-check failing samples based on comparison of phenotype and  
128 sequencing data.<sup>14</sup> The genomic region of CHCHD10 (NCBI Reference Sequence:  
129 NG\_034223.1) was isolated from the VCFs and variants were annotated using  
130 Variant Effect Predictor.<sup>15</sup>

### 131 **Burden Testing**

132 Gene regions were isolated based on their canonical transcripts in the Ensembl  
133 database (<http://www.ensembl.org>). Within these regions, single nucleotide variants  
134 (SNVs) that were annotated as missense or loss-of-function mutations with a minor  
135 allele frequency (MAF) <1% in the control population and public databases were  
136 selected for burden testing. Burden testing on cases and controls was performed  
137 using bidirectional sequence kernel association test (SKAT) together with SKAT-O to  
138 account for an unidirectional effect, more likely in the case of mainly damaging  
139 variants.<sup>16</sup> Association tests were corrected for population stratification using the first  
140 10 principal components. Additionally, 100.000 permutations were performed with  
141 SKAT-O to obtain the empirical SKAT-O p-value. Statistical analyses were carried  
142 out using R software (<http://www.r-project.org>).

143

## 144 **Results**

145 To investigate variants in CHCHD10, we analyzed all rare, non-synonymous SNVs in  
146 the whole-genome sequencing data of 4,365 ALS ( $\pm$  FTD) samples together with  
147 1,832 unaffected controls. We identified seven SNVs in ALS cases, three of which  
148 were not previously reported (Table 1). Screening of controls revealed that only three  
149 out of these seven variants were case-specific, as the other four variants were also  
150 found in controls. Additionally, one control-specific SNV was identified.

151

### 152 **No increased burden of rare variants**

153 Neither the SKAT nor the SKAT-O association tests showed a significant increased  
154 burden of rare non-synonymous variants in CHCHD10 among ALS patients ( $P =$   
155 0.88 and  $P = 1.00$ , respectively; Table 2). As a positive control, we tested three other  
156 genes (SOD1, FUS and TARDBP), which are known to harbor rare pathogenic SNVs  
157 in ALS.<sup>17</sup> These genes did yield a significant SKAT-O association statistic ( $P =$   
158 0.008,  $P = 0.02$ , and  $P = 0.03$  respectively; Table 2).

159

### 160 **Additional clinical information on carriers**

161 Only three rare missense mutations in CHCHD10 were specific to ALS cases (Table  
162 2). The previously unreported p.Arg11Gly mutation was identified in a single female  
163 ALS case from the United States without cognitive involvement and a negative family  
164 history for ALS or dementia. We identified three cases with the previously reported

165 p.Arg15Leu variant: one Dutch and two American cases, one of which was already  
166 included in the previous study by Johnson et al. (ND11809).<sup>5</sup> Although both  
167 American cases had a positive family history, the additional Dutch ALS patient did  
168 not have a family history of ALS or dementia. Similar to previously described  
169 carriers, the clinical phenotype in this patient was characterized by very slow  
170 progression with both upper and lower motor neuron involvement, a long diagnostic  
171 delay of two years and a disease duration of over eight years after onset.<sup>5, 6, 18</sup>  
172 Interestingly, besides motor neuron disease, this patient presented with an atypical  
173 phenotype including deafness, weakness of the proximal upper extremities and low  
174 tendon reflexes. Unfortunately, no muscle biopsies were performed. The third case-  
175 specific mutation (p.Pro80Leu), previously reported in an Italian ALS patient with an  
176 abnormal muscle biopsy (COX deficiency), was found in a Belgian ALS patient.<sup>7</sup> This  
177 patient also presented with an atypical myopathy-like clinical phenotype with  
178 proximal lower limb weakness and high serum creatine kinase levels (up to 1800  
179 U/l). The clinical features at the time of presentation prompted the neurologist to  
180 request a muscle biopsy, which showed neurogenic atrophy, but without  
181 histochemical analysis for COX.

182

## 183 **Discussion**

184 CHCHD10 was proposed to be a new candidate gene for ALS following the initial  
185 report of a p.Ser59Leu variant, which was detected in a family with a complex  
186 phenotype including ataxia, myopathy, dementia and a progressive motor neuron  
187 disease resembling ALS.<sup>3</sup> Subsequently, several studies screened for CHCHD10  
188 mutations in ALS patients and healthy controls and claimed pathogenicity for multiple



189 rare missense variants.<sup>4-6</sup> 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.<sup>10</sup>

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).

213 In the absence of linkage or a statistically significant burden test, all variants  
214 that are solely observed in a single case do not meet criteria for pathogenicity.<sup>8</sup> Only  
215 variants that occur in multiple unrelated cases (and no controls) are potentially more  
216 interesting. Together with previous reports, only six CHCHD10 variants have met this  
217 criterion (Table 4). Some of these variants are already listed as pathogenic in public  
218 databases such as ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>) despite the fact  
219 that other criteria for establishing pathogenicity were often not investigated.

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

226 Similar to previous observations, the most frequent rare non-synonymous  
227 SNV in our dataset was the heterozygous p.Pro34Ser, which was present in 37  
228 cases (0.85%) as well as 15 control samples (0.82%) (corrected  $\chi^2(1) = 0.00$   $P =$   
229  $0.98$ ). Despite initial reports of possible pathogenicity of this variant in pure ALS  $\pm$   
230 FTD, our data adds to the increasing evidence that the p.Pro34Ser mutation in  
231 CHCHD10 is probably not pathogenic.<sup>19-22</sup> Recent in vitro studies still support  
232 p.Pro34Ser pathogenicity as similar cellular pathology between CHCHD10<sup>S59L</sup> and  
233 CHCHD10<sup>P34S</sup> mutant cell lines was shown.<sup>23</sup> Despite the in vitro findings, the fact  
234 that the p.Pro34Ser variant is as common in ALS patients as in the general  
235 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.<sup>7, 24</sup> 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.<sup>20, 25-27</sup> 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.<sup>5, 6, 18, 28</sup> 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

260 penetrance).<sup>6</sup> Here, we report two new carriers: one in the Dutch cohort and one in  
261 the US cohort (the other US carrier has already been reported). Although limited, the  
262 available clinical data for these patients seems to be similar to that reported in other  
263 carriers (predominant lower-motor neuron symptoms and slow disease progression)  
264 with some atypical symptoms in one patient (bilateral hearing loss and proximal  
265 onset).<sup>6, 18</sup> This again points towards a distinct ALS-like clinical phenotype. However,  
266 the percentage of ALS cases which might be explained due to this variant is 0.1%  
267 (9/6,797 non-overlapping cases) making it a possibly pathogenic but very rare  
268 CHCHD10 variant in motor-neuron disease.

269         The association of CHCHD10 mutations in motor-neuron disease resembling  
270 ALS is further illustrated by the c.197G>T (p.Gly66Val) variant, which was originally  
271 described in a Finnish familial ALS patient with slowly ascending progressive motor  
272 neuron disease. This variant was later shown to cause a lower motor neuron  
273 phenotype without upper-motor neuron or cognitive involvement as it was identified  
274 in 75 Finnish carriers with hereditary, late onset spinal motor neuropathy (SMAJ),  
275 Charcot-Marie Tooth disease Type 2 or both.<sup>6, 29-31</sup>

276         Overall, there seems to be potential evidence for the involvement of  
277 CHCHD10 in neurodegenerative diseases, particularly in combination with clinical  
278 features that suggest mitochondrial dysfunction, such as myopathy or hearing-loss.  
279 In the case of pure ALS however, our results indicate that rare genetic variants in  
280 CHCHD10 can be detected in both cases and controls at similar frequencies.  
281 Therefore, CHCHD10 variants seem to be a far less prevalent cause of pure ALS  
282 than previously suggested.<sup>5</sup> This study shows that routine testing for  
283 CHCHD10 variants in pure ALS is not recommended and illustrates the importance

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

286

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317

## 318 **Author Contributions**

319 Contributing authors are listed below. The full list members of the Project MinE ALS  
320 Sequencing Consortium with affiliations and contributions are listed in  
321 Supplementary Table 1.  
322 Gijs H.P. Tazelaar,<sup>#</sup> Wouter van Rheenen,<sup>#</sup> Sara L. Pulit, Rick A.A. van der Spek,  
323 Annelot M. Dekker, Matthieu Moisse, Russell McLaughlin, William Sproviero, Kevin  
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326 Jonathan D. Glass, Philip Van Damme, Orla Hardiman, John E. Landers, Leonard H.  
327 van den Berg, Jan H. Veldink.  
328 <sup>#</sup>these authors contributed equally  
329

330 **Conflicts of Interest**

331 Nothing to report.

332

333

334  
335

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

336

337 **Table 1. CHCHD10 Variants in Project Mine** Overview of rare (MAF <1%) single  
338 nucleotide variants, functionally annotated as missense or loss of function in a total  
339 of 4,365 ALS and 1,832 control samples with the genomic location, location in  
340 transcript NM\_213720.1 and predicted amino acid change, allele counts and  
341 corresponding minor allele frequencies (MAF) together with the MAF of the  
342 European population in the ExAC database.

343



344  
345

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

346  
347

348 **Table 2. Burden Testing** Results of burden test analysis using SKAT and SKAT-O  
349 association testing on rare (MAF<1%) non-synonymous single nucleotide variants in  
350 CHCHD10 and known ALS genes. Nvar indicates the number of SNVs which were  
351 taken into account for association testing.

352

353  
354

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

355  
356

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

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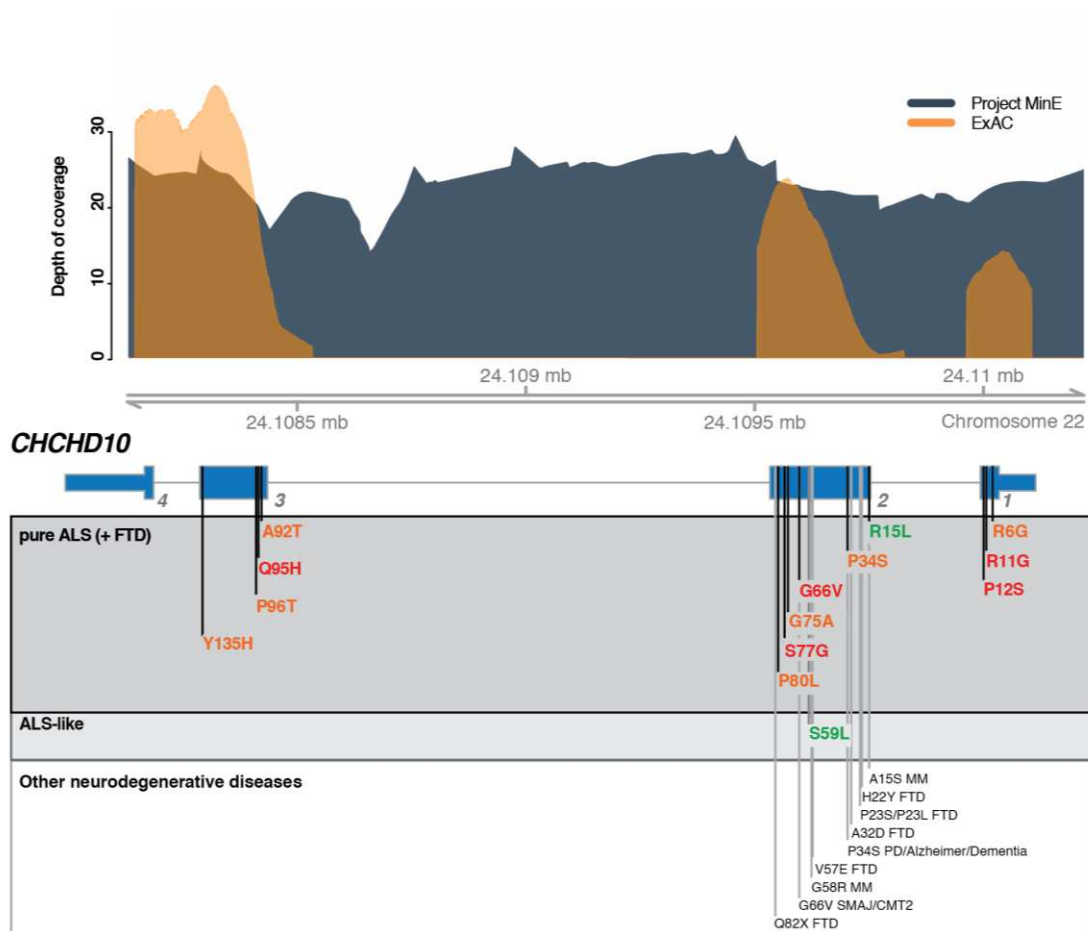
Variants:	Project Mine		Previous reports		Segregation in Pedigree(s):	ClinVar
	ALS	Controls	ALS	Controls		
p.Arg15Leu	3	0	7	0	Yes	Conflicting
p.Pro34Ser	37	15	20	25	No*	Pathogenic
p.Ser59Leu	0	0	2	0	Yes	Pathogenic
p.Pro80Leu	1	0	4	0	Unknown	Pathogenic
p.Pro96Thr**	2	2	6	3	Unknown	Unknown
p.Tyr135His	3	1	1	0	Unknown	Unknown
Total screened	8730	3664	7560	6604		
No overlap	<b>5140</b>	<b>2778</b>				
* In a pedigree with FTD <sup>22</sup> **Allele counts were not provided in all reports <sup>25</sup>						

367  
368

**Table 4. Non-synonymous CHCHD10 variants in multiple ALS / FTD cases.**

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

375



377

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

379 Overview of rare non-synonymous variants in ALS and other neurodegenerative

380 diseases and their exonic location in CHCHD10. The top panel shows depth of

381 coverage of CHCHD10 in the ExAC public database (orange) and Project Mine

382 whole-genome sequencing data (blue-grey) (<http://databrowser.projectmine.com>).

383 The grey panel shows all variants reported in pure ALS  $\pm$  FTD; variants in green

384 were present in multiple seemingly unrelated cases and absent in controls, orange

385 variants were identified in both cases as well as controls and red variants were found

386 in a single ALS case. The light grey panel shows variants reported in a more

387 extensive phenotype that includes motor neuron disease. The bottom panel shows

388 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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