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Screening for C9ORF72 repeat expansion in FTLD

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Abstract

In the present study we aimed to determine the prevalence of *C9ORF72* GGGGCC hexanucleotide expansion in our cohort of 53 FTLD patients and 174 neurologically normal controls. We identified the hexanucleotide repeat, in the pathogenic range, in 4 (2 bv-FTD and 2 FTD-ALS) out of 53 patients and one neurologically normal control. Interestingly, two of the *C9ORF72* expansion carriers also carried two novel missense mutations in *GRN* (Y294C) and in *PSEN-2* (I146V). Further, one of the *C9ORF72* expansion carriers, for whom pathology was available, showed amyloid plaques and tangles in addition to TDP-43 pathology. In summary, our findings suggest that the hexanucleotide expansion is probably associated with ALS, FTD or FTD-ALS and occasional comorbid conditions such as Alzheimer's disease. These findings are novel and need to be cautiously interpreted and most importantly replicated in larger numbers of samples.

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Disclosure statement

All authors disclose no actual or potential conflicts of interest. All human study protocols were approved by the ethics committee of local institutions. An informed consent was obtained from the persons with the power of attorney for the patient.

Keywords

FTLD; bv-FTD; FTD-ALS; *C9ORF72*; *GRN*; *PSEN-2*; Alzheimer's disease

Introduction

Frontotemporal lobar degeneration (FTLD, OMIM #600274) is the second most common cause of presenile dementia. It is characterized by progressive degeneration of the frontal and anterior temporal lobes of the brain leading to wide range of clinical symptoms including changes in personality and behavior, language impairment, and cognitive dysfunction (reviewed in Ferrari et al. 2011). Approximately 15% of FTLD patients develop symptoms of motor neuron dysfunction (Lomen-Hoerth et al., 2002). The co-occurrence of FTLD and MND in familial cases suggests that FTLD represents a spectrum of neurological disorders with complicated clinical, pathological and genetic etiology. The most common form of FTLD pathology is FTLD-TDP with TAR DNA-binding protein-43 (TDP-43) immunoreactive neurons, which is also deposited in neurons of ALS patients. This pathological finding links FTLD and ALS (Neumnann et al., 2006; Arai et al., 2006).

To date, mutations in the progranulin gene (*GRN*) are considered the most prevalent and encompass phenotypes from bvFTD to CBS. The common pathology in *GRN* mutation carriers is TDP-43 positive inclusions. Mutations in the *TDP-43* gene however, are usually associated with the ALS phenotype (Corrado et al. 2009) and rarely found in FTLD (Borroni et al. 2009) or CBS (Huey et al. 2011). The causal link between the mutations in *GRN* gene and TDP-43 pathology is yet to be established. Mutations in the other FTLD candidate genes, *MAPT*, *VCP*, *CHMP2B*, *PSEN1*, and *PSEN2* explain only very small number of FTLD patients and there is no report of ALS or MND among those mutation carriers.

Linkage studies of cases with ALS, FTD, FTD-ALS with type 2 TDP-43 pathology had suggested a locus on chromosome 9p (Boxer et al., 2011; Morita et al., 2006; Vance et al., 2006) although it was not clear if the GWA and the linkage studies identified the same locus on 9p. Mok et al. in 2011 suggested a common founder for FTLD and ALS on chromosome 9, based on the original Finnish association study reported by Laaksovirta et al in 2010. The identification of hexanucleotide GGGGCC repeat expansion in the non-coding region of *C9ORF72* in families linked to 9p21 (DeJesus-Hernandez et al., 2011; Renton et al., 2011) suggested this expansion as a possible cause of FTLD and ALS.

In the present study we screened 53 patients and 174 neurologically normal controls for the expansion of the GGGGCC hexanucleotide. Our cohort encompasses a wide range of the FTLD spectrum which may prove useful in establishing a phenotype/genotype correlation within the FTLD spectrum. In the present study, we identified 4 out of 53 patients and one neurologically normal control who had donated a sample to the Coriell institute who carried the hexanucleotide repeat in the apparent pathogenic range.

Patients and methods

Patients

The study population comprised a sub-group of patients which have been previously described (Huey et al. 2011), consisting of 27 probable bv-FTD, 9 possible bv-FTD, 6 PPA-PNFA, 2 PPA-semantic, 4 FTD-ALS, 4 AD, and 1 MSA diagnosed using appropriate criteria (Neary et al., 1998; Raskovcky et al., Brain 2011; McKhann et al., 1984; Gilman et al., 2008). A full neurological and neuropsychological evaluation was performed for all patients. Table 1 presents a brief family history of all patients.

Genetic screening

Blood samples from index patients were collected in accordance with the local Institutional Review Board guidelines and informed written consent form was obtained. The DNA of all patients was screened for *MAPT*, *PGRN*, *FUS*, *TDP43*, *PSEN1*, *PSEN2*, *APP* and *CHMP2B*. In order to screen for hexanucleotide expansions in the *C9ORF72* gene, we performed the experiments as described in Renton et al. (2011). Although this method is not able to determine the exact number of repeats, it can detect repeat numbers of maximum ~ 60. This method however, can discriminate the normal repeat range detected in the normal population (0-20) from the higher mutated range (≥30). Renton et al. (2011) detected a pathogenic repeat number of ~250 repeats in a carrier whose fluorescence in situ hybridization (FISH) analysis verified the repeat expansion. DeJesus-Hernandez et al. (2011) used a different method to amplify the repeat region of *C9ORF72* and for the verification of repeat size they subsequently performed Southern blot analysis. They estimated the number of repeats between 700-1600. As the goal of our study was solely to determine if our cohort has repeat numbers above the range of normal controls, we screened the DNA of 53 patients and 174 neurologically normal controls from Coriell Institute (NDPT 098 and 099). The mutated alleles were considered at the length of 30 repeats and above (Renton et al., 2011). The DNA of these patients had been genotyped on the illumina infinium platform as part of the ongoing international FTD-GWAS consortium project. The genotypes of 42 SNPs which were used to build the chromosome 9p21 haplotype in Finnish population (Laaksovirta et al., 2010) were derived from the GWAS data for the 53 patients to determine if the carriers of the expansion also harbor the same ancestral haplotype as the Finnish carriers of hexanucleotide expansion.

Results

In our cohort we identified 4 out of 53 patients (FTD158, FTD198, FTD211 and FTD223) and one out of 174 neurologically normal controls (Coriell plate NDPT099 - ND07551) with repeat expansion in the pathogenic range (≥30) (Figure 1). Among the carriers of the hexanucleotide expansion, one patient (case 1 FTD158) carries a novel *GRN* mutation Y294C (Figure 2A) and another patient (case 4 FTD223) carries a novel mutation in *PSEN2* I146V (Figure 2B). None of these mutations have been reported previously and there is no functional data available regarding pathogenicity of these variants. Due to lack of informative family members, we could not examine the segregation of these mutations with the disease. The *in silico* analysis by PolyPhen-2 software (PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/) predicted both variants to be probably damaging.

We extracted the genotypes of 42 SNPs of the original Finnish haplotype by Laaksovirta et al 2010 to determine if our patients harbor the original risk haplotype on chr9p21 for FTD-ALS (rs3849942, rs1330921, rs10121765, rs1110264, rs1110155, rs2150336, rs2225389, rs1161680, rs2120718, rs2589054, rs10812596, rs1058326, rs944404, rs765709, rs1316679, rs4406503, rs10511817, rs725804, rs10511816, rs1444533, rs1822723, rs4879515, rs895023, rs868856, rs7046653, rs2440622, rs1977661, rs903603, rs10812610, rs2814707, rs12349820, rs10122902, rs10757665, rs1565948, rs774359, rs2282241, rs1948522, rs1982915, rs2453556, rs702231, rs696826, rs2477518). Our genotyping (Supplementary Table 1) revealed that the complete risk haplotype does not exist in any of our patients. However, Renton et al 2011 reported that SNP rs3849942 is a surrogate marker for the risk haplotype in the populations that they studied. Our 4 patients with expansions carry the risk allele A (rs3849942) and 20 out of 49 of the rest of patients without expansion carry that risk allele as well. Of note, in our patients series there were two individuals diagnosed with FTD-MND, samples FTD134 and FTD163 (Table 1). Patient FTD134 carries neither the expansion nor the risk allele A (rs3849942), while FTD163 does not carry the expansion but the risk allele.

The family history of the patients who carry the hexanucleotide repeat expansion can be seen in more detail in the pedigrees (Figure 3). A full clinical description of these four patients can be found in the supplementary clinical data.

Discussion

Our cohort of FTLD patients was recruited from around the country and was not selected on the basis of family history, known familial mutations, or presence of MND. This sample is likely representative of the general U.S. FTLD population. In the quest to identify the genetic cause of FTLD in our cohort, we screened 53 of our patients for the newly identified locus on chromosome 9p21 (DeJesus et al., 2011; Renton et al., 2011). Patients were referred to NIH primarily with symptoms of frontotemporal dementia and some had developed accompanying neuropsychiatric or motor neuron symptoms as well (Table 1). We identified hexanucleotide expansion of over 30 repeats in 4 patients of which two have a diagnosis of FTD-ALS and two of bv-FTD. As our mutation finding did not reach the statistical significance of the original publications by DeJesus-Hernandez et al. and Renton et al., we aimed to verify the existence of the risk haplotype in our population (Supplementary Table 1). However, neither the risk alleles of the 42 SNPs of the original Finnish risk haplotype (Laaksovirta et al., 2010), nor the phenotypes of the mutation carriers showed a strong correlation to the hexanucleotide expansion compared to those who did not carry the mutation. Among our 53 patients, no one carries the exact same Finnish risk haplotype. Considering the fact that our patients are all recruited in the U.S. and have diverse ethnic background this outcome is not unusual. The haplotype can be explained as a possible genetic background for the carriers of the expansion and not necessarily associated with the hexanucleotide expansion. The phenotype of our cohort was also diverse. As reported previously (DeJesus-Hernandez et al., 2011 Renton et al., 2011; Gliselnick et al., 2011; Troakes et al., 2011), the expansion is detected mainly in FTD-MND cases. As evident in Tables 1 and 2 we had overall 4 patients with FTD-ALS or FTD-MND (of which only two, FTD211 and FTD223, carried the expansion) and 2 bv-FTD cases with family history of ALS (FTD183 and FTD218) who did not carry the hexanucleotide expansion. Additionally, we identified the expansion of more than 45 in one neurologically normal control (see Supplementary clinical data). These findings prompt us to have a more critical view to understand whether the *C9ORF72* hexanucleotide repeat expansion is the pathogenic cause of the disease or merely a risk factor contributing to the susceptibility to the disease. As we have previously shown (Huey et al., 2011) in cases such as *FUS* gene, the variants which were originally reported as pathogenic in ALS cases were later found also in neurologically normal controls. Furthermore, we replicated an interesting finding by Murray et al (Table 2): the *C9ORF72* expansion carriers may be more likely to present with memory and psychiatric symptoms rather than typical FTLD symptoms. One of our *C9ORF72* expansion positive patients presented with paranoia as his predominant symptom and two others demonstrated significantly impaired memory. The one patient whose autopsy report is available (FTD211) showed amyloid plaques and tangles in addition to TDP-43 pathology (Dr. Ghetti personal communication). Murray et al. also describes one expansion case with both TDP-43 and AD pathology. This suggests that the hexanucleotide expansion may also coexist with forms of dementia other than ALS, FTLD, or FTD-ALS. Although this is only a single case it raises the possibility that some cases of clinical and pathologic TDP-43 positive AD may be associated with *C9ORF72* expansions. This association is proven best through screening of pathologically confirmed AD cases which would link *C9ORF72* expansions to AD as a cause of the disease, if confirmed.

Lastly, it is notable that we found two missense mutations, Y294C in *GRN* and I146V in *PSEN2*, in two of our hexanucleotide expansion carriers (FTD158 and FTD223) which had not been reported previously. There are several possible interpretations of this finding: **1**.

These missense mutations are benign and the disease is caused by the *C9ORF72* expansion. However, it seems unlikely that this is the case because we found the expansion in a neurologically normal control and also because, although these two missense mutations are novel and have not been previously reported, *in silico* analysis by Polyphen 2 predicts highest level of pathogenicity for these two variants. Moreover, while *GRN* missense mutations have not been considered as a common cause of FTLD, *PSEN2* missense mutations have been indisputably shown to be pathogenic. **2**. The *GRN* and *PSEN2* missense mutations are the primary pathogenic causes of the disease in these two FTD patients and the *C9ORF72* expansion contributes to the disease as a risk factor, increasing the susceptibility to the disease. **3**. These are two complex cases where two comorbid conditions are caused by two separate lines of genetic mutations, namely the missense mutations on one hand and *C9ORF72* expansion on the other hand. If this is the case, the *GRN* mutation would most probably lead to TDP-43 pathology and the *PSEN-2* mutation to the amyloid plaques. These two patients will probably have an additional pathology due to *C9ORF72* expansion which is yet to be identified.

To conclude, in this study we report the presence of hexanucleotide repeats in two bv-FTD cases, two FTD-ALS cases (one of which showed, at autopsy, FTLD-TDP type B and AD pathology), and one neurologically normal control from Coriell cell repository. Our findings suggest that the hexanucleotide expansion is probably associated with ALS, FTD or FTD-ALS and occasional comorbid conditions such as Alzheimer's disease as evident in our pathologically confirmed case 3 (FTD211). These findings are novel and need to be cautiously interpreted and most importantly replicated in larger numbers of samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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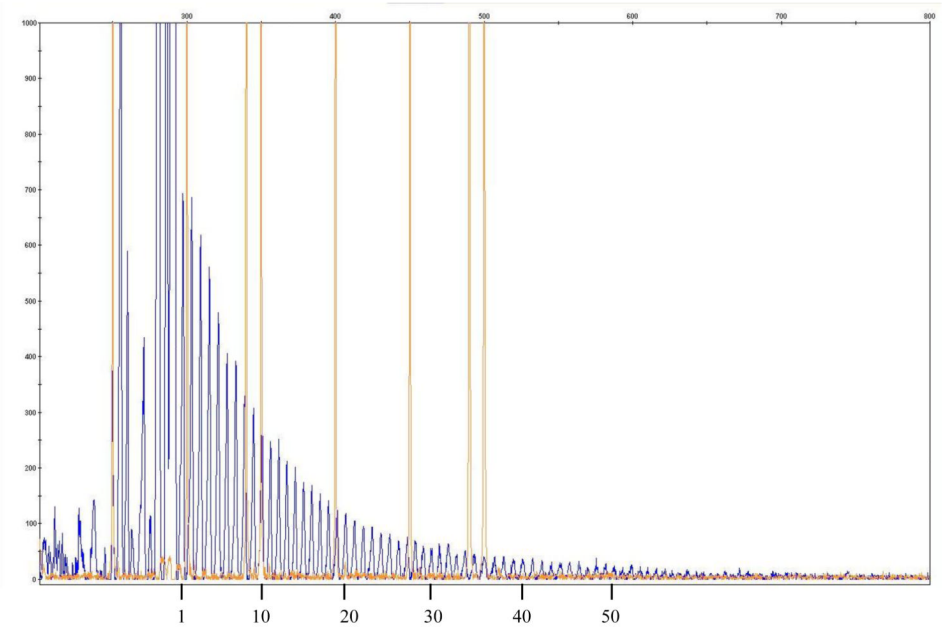
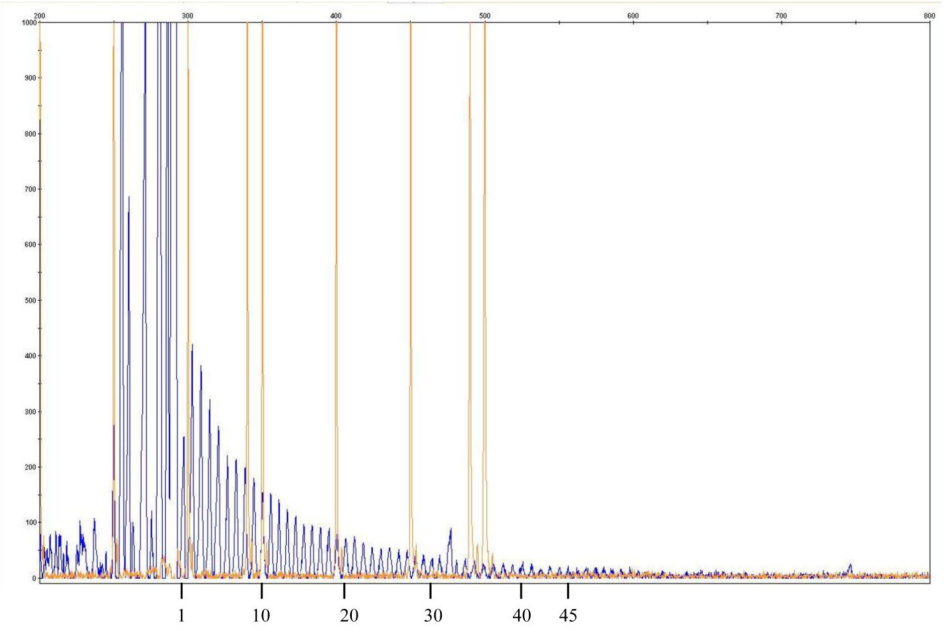
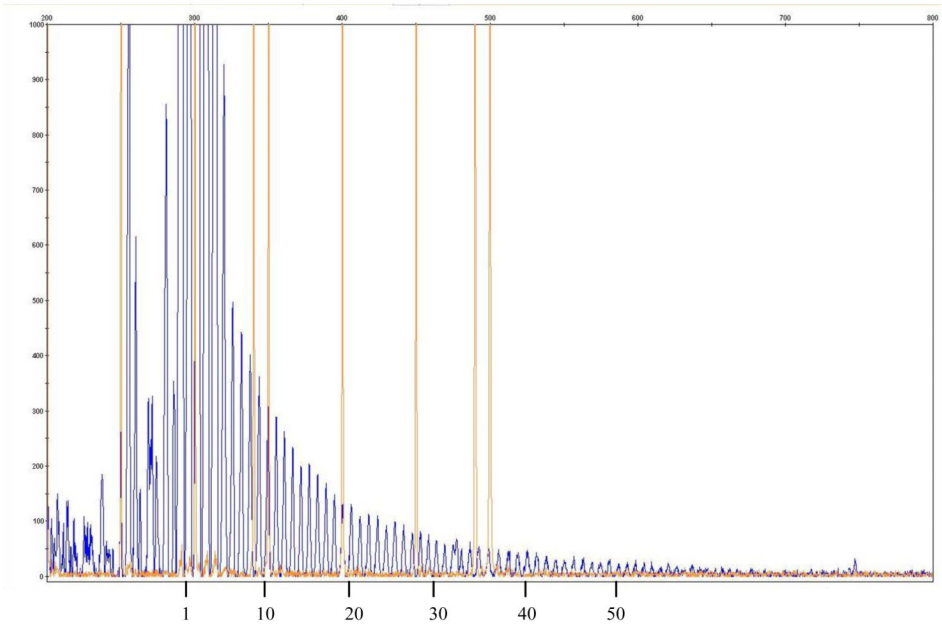
A. FTD-158 – chr9 repeats > 50



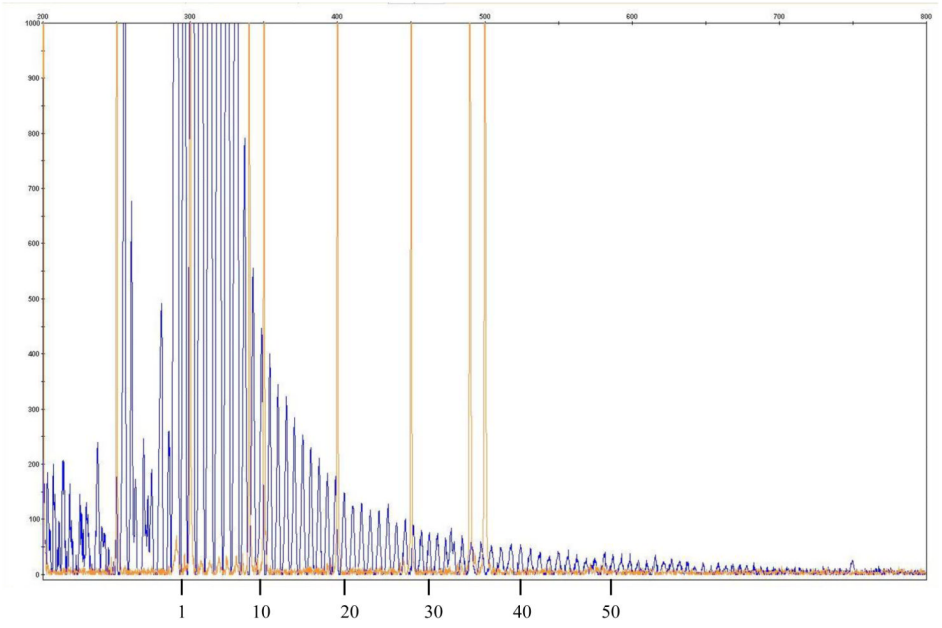
B. FTD-198 – chr9 repeats > 45



C. FTD-211 – chr9 repeats > 50



D. FTD-223 – chr9 repeats > 50



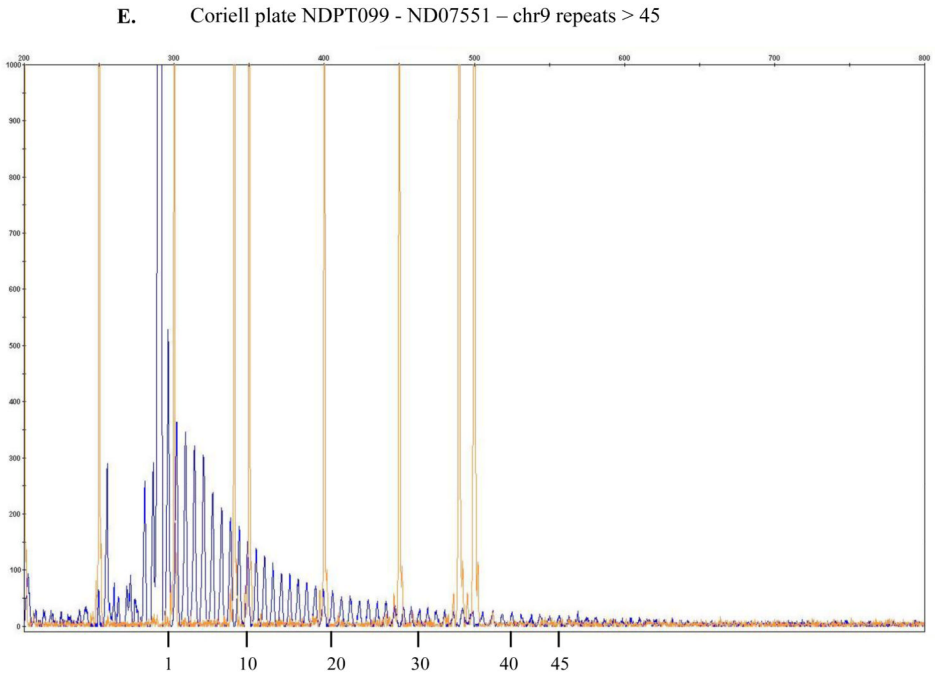


Figure 1.

The repeat-primed PCR reactions were run on ABI3730 DNA Analyzer .The results were analyzed & visualized using Gene Mapper software. Fluorescence intensities are shown in the vertical axis: the size marker is identified by orange vertical lines and the hexanucleotide repeats that extend beyond the 300 bp marker are marked in blue. The range of > 30 repeats is considered the threshold suggesting presence of expansion even though a direct and precise relatedness between this method and the actual repeat size has not been established yet being, as such, simply predictive of probable repeat expansion. In here, counts of approximately > 45 or > 50 repeats are depicted for, respectively, samples FTD-158 (**A**), FTD-198 (**B**), FTD-211 (**C**), FTD-223 (**D**) and for Coriell neurologically normal control ND07551 (**E**).

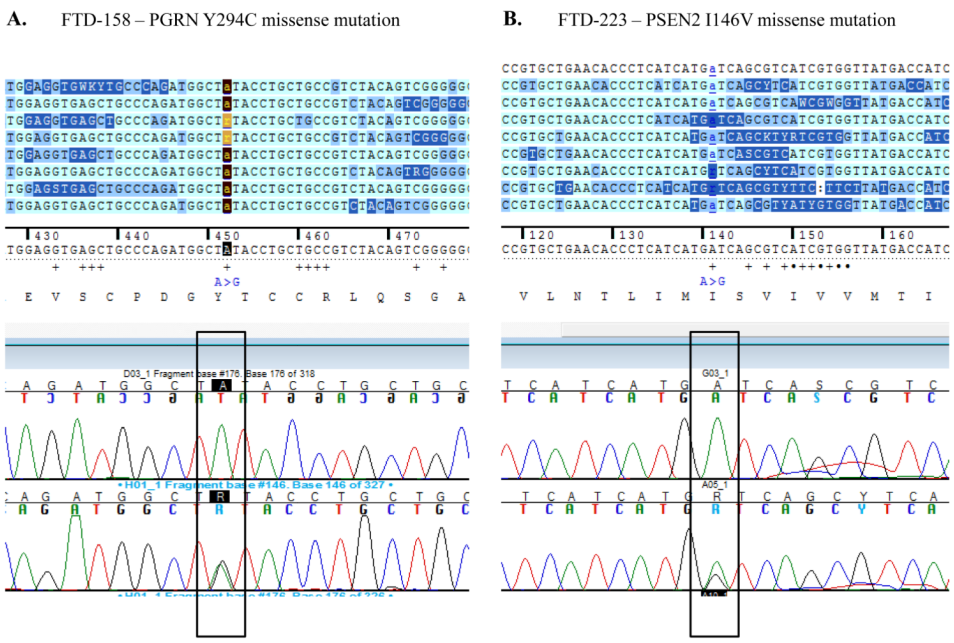
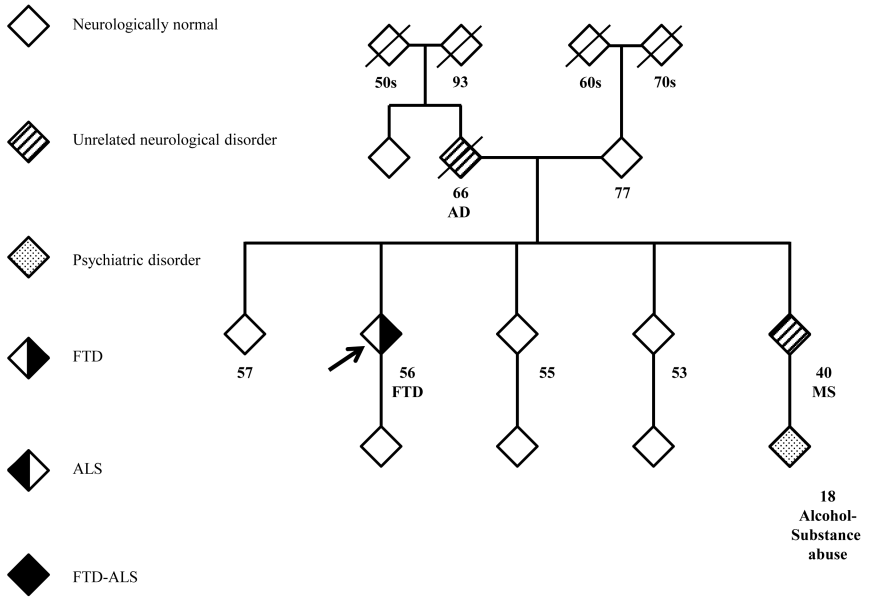


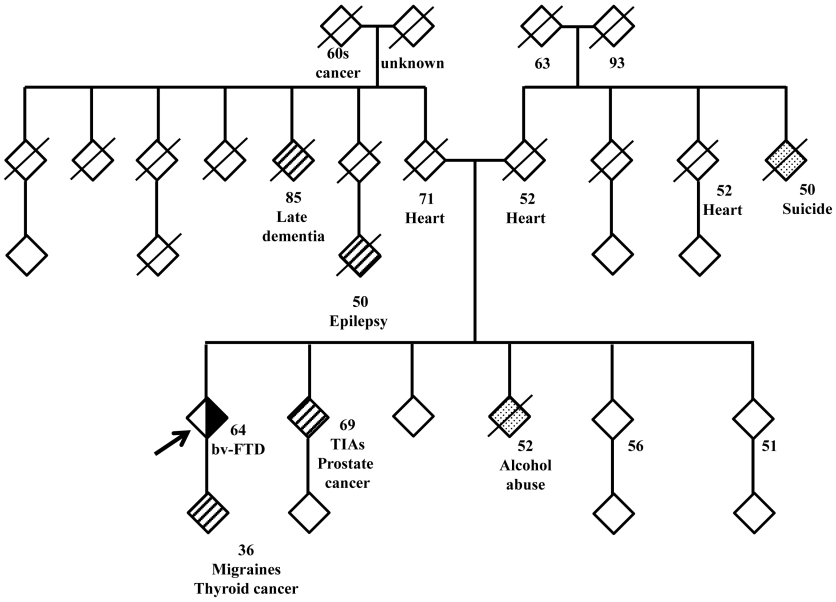
Figure 2.

Electropherograms of the two novel missense mutations found in *PGRN* (**A**) and in *PSEN2* (**B**) in two of the hexanucleotide repeat carriers, respectively individuals FTD-158 (A) and FTD-223 (B). Both missense mutations have not been previously reported. After *in silico* analysis using Polyphen 2 software, both mutations have been predicted to be probably damaging.

Case 1 FTD158



Case 2 FTD198



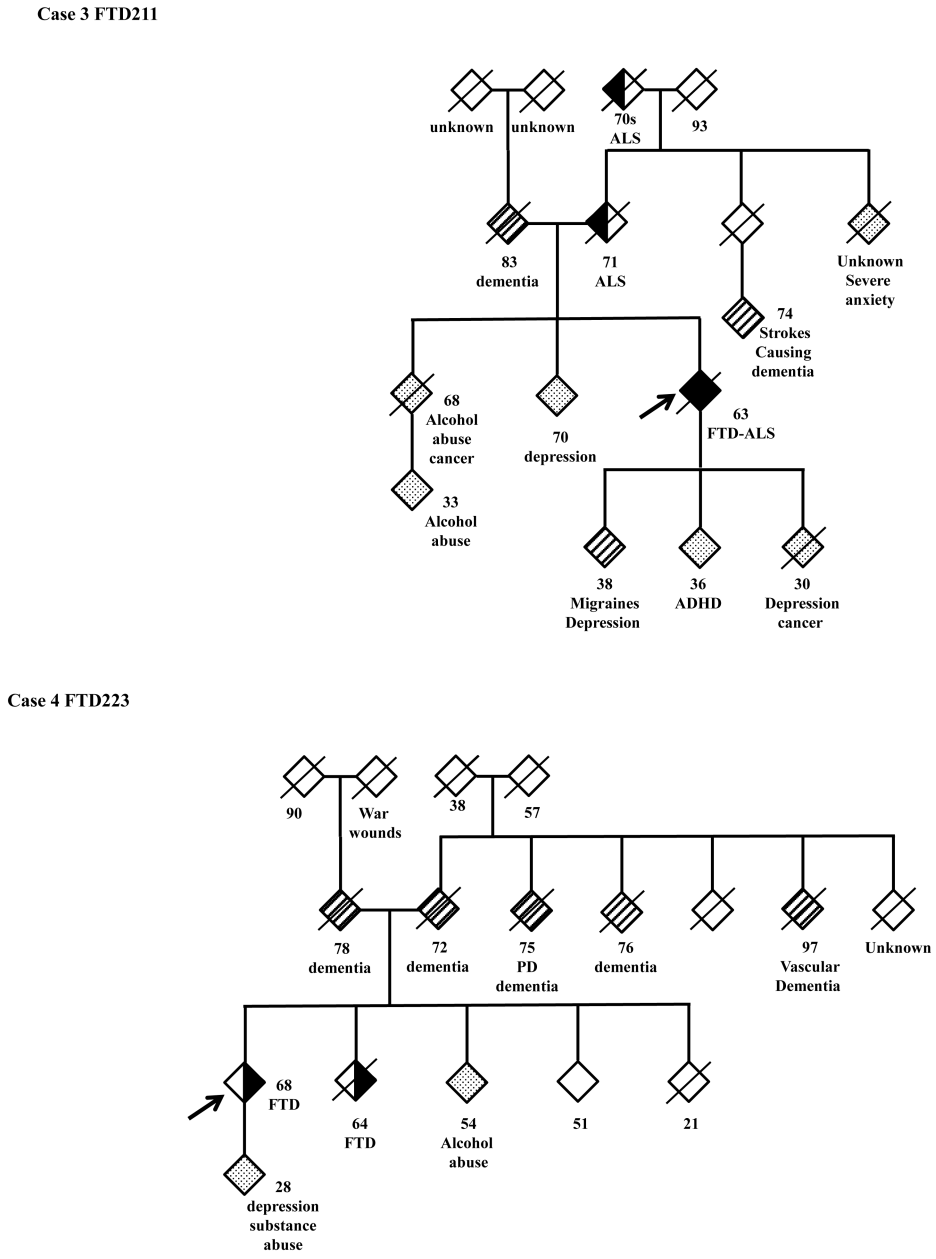


Figure 3.

Pedigrees of the patients carrying the C9ORF72 hexanucleotide expansion. 3A: FTD158, 3B: FTD198, 3C: FTD211, 3D: FTD223.

FTD: Frontotemporal Dementia; ALS Amyotrophic Lateral Scelerosis; AD: Alzheimer's Disease; MS: Multiple Sclerosis; TIA: Transient Ischemic Attack; ADHD: Attention Deficiency Hyperactivity Disorder; PD: Parkinson's Disease.

Table 1

Samples IDs with corresponding diagnosis, number of hexanucleotide expansion and a brief family history of neurological disorders for each patient. Diagnoses are described following Rascovsky et al., 2011.

Patient ID	Diagnosis	Hexanucleotide Repeats #	Family history of neurological disorders
158	Probable bv-FTD	> 50	Father - AD; Brother - MS
198	Probable bv-FTD	> 45	Brother - TIA; Brother - alcoholism; Daughter - migraines; Paternal aunt - dementia
211	FTD-ALS	> 50	Mother - ALS; Brother - alcoholism; Father - dementia; Brother/Son - depression; Daughter - migraines, depression; Son - anoxic at birth
223	FTD-ALS	> 50	Mother - dementia; Father - dementia ; Sister - FTD ; Brother - alcoholism ; Son - depression, drug use
81	Probable bv-FTD	1	Father - died of dementia at 77
83	AD	7	Paternal grandfather - dementia
85	FTD-PPA	7	Father -vasc. dementia died at 68
88	FTD-PPA	14	Sister - PD
89	Probable bv-FTD	8	Mother - LOAD; Paternal grandmother - dementia died at 89
90	Probable bv-FTD	4	Father - CVA; Nephew - OCD
95	SD	4	Maternal aunt - dementia
96	Probable bv-FTD	~ 25	Father - AD onset 58; Maternal uncle - schizophrenia
97	FTD-PPA	3	Son - schizophrenia
98	Possible FTLD	15	Sister CVA. O/w no +FH
99	Probable bv-FTD	10	Father - PD died at 85; Mother dementia, brain tumor; 3 aunts - dementia; Maternal grandmother - dementia
101	Probable bv-FTD	3	Son - schizophrenia, drug abuse; Daughter - schizophrenia; Sister &Brrother - drug abuse
103	SD	5	Maternal grandmother - CVA; Maternal aunt - CVA
107	Probable bv-FTD	1	Paternal uncle - unknown mental illness
111	Possible FTLD	2	Sister - Korsakoff syndrome; Mother - OCD, depression; Sister -alcoholism; Twin brother - alcoholism
114	FTD-PPA	2	Father - AD, PD died at 63; 2 brothers - schizophrenia
115	Probable bv-FTD	6	Father - dementia onset at 72 paternal grandmother Parkinson’s and memory problem died in her 70”s
118	Probable bv-FTD	6	Father - AD; Mother - AD, depression both died in their early 90’s
123	Probable bv-FTD	8	Brother - learning disabilities, alcoholism; Sister - eating disorder, depression
124	Probable bv-FTD	7	Father - PD; aunt AD died at 79
127	MSA	1	Mother - AD; Brother - CVA; Brother - brain cancer

Patient ID	Diagnosis	Hexanucleotide Repeats #	Family history of neurological disorders
128	Probable bv-FTD	3	Father - OCD; Mother - Mental disorder NOS
134	FTD-MND	1	
136	Probable bv-FTD	2	Mother - dementia at 87; maternal uncle dementia
139	FTD-PPA	10	Mother - depression, alcoholism
143	Probable bv-FTD	17	Daughter - OCD, depression
144	Probable bv-FTD	5	
163	FTD-MND	9	Paternal uncle - dementia, alcoholism
176	FTD-PPA	2	Mother - depression; Daughter - ADD
177	AD	3	Mother - LOAD; Father - alcoholism; Son - alcoholism
183	Possible FTLD	2	Father PTSD & depression; mother DLB, mutism; mat grandfather PD & stroke; maternal uncle DLB; 2 paternal aunts with MND
194	Probable bv-FTD	3	Sister - dementia; Father - stroke; Mother - depression ; Son - depression/anxiety
195	Probable bv-FTD	6	Mother - action tremor; Paternal grandmother - dementia; maternal great grandmother dementia
199	Possible FTLD	2	Father - dementia ; Daughter - seizures ; Maternal grandmother - dementia
200	Probable bv-FTD	6	Daughter - ADD, anxiety; Son - substance abuse
201	Possible FTLD	4	Father- dementia in his 60’s, stroke; Sister - dementia; Brother - stroke; Brother - epilespy, stroke; Son - epilepsy, mental illness, grandfather dementia in his 60’s
203	Probable bv-FTD	3	Paternal grandmother - late onset dementia at 91; Mother - MS; maternal grandmother Parkinson’s onset at 78, maternal great aunt and uncle late onset AD
205	Possible FTLD	10	2 Sisters - anxiety; Sister - PSP; Daughter - eating disorder; Daughter - anxiety, depression; Daughter - schizophrenia; Son/2 Daughters - depression
207	Possible FTLD	7	Siblings tremor, anxiety, alcoholism, substance abuse, mother died at 83 had dementia and anxiety; father died at 79 had Parkinson’s and anxiety.
209	Probable bv-FTD	7	Father - stroke ; Brother - born w/hole in skull; Daughter - seizure disorder
210	Possible FTLD	7	Maternal grandfather - dementia
212	Probable bv-FTD	5	
215	Possible FTLD	2	Mother - AD ; Father - dementia ; Brother - depression ; Son - arthrogyrosis
216	Possible FTLD	9	Sister - mild stroke, depression
217	Probable bv-FTD	2	father died at age 82 no dementia but paternal grandfather died of dementia at 81
218	Probable bv-FTD	~ 25	Mother - dementia; Son - ALS; Maternal aunt - AD

Patient ID	Diagnosis	Hexanucleotide Repeats #	Family history of neurological disorders
219	Probable bv-FTD	2	Daughter - anx, dep, neurocardiogenic syndrome ; Brothers - depression ; Sister - anx, dep, ADHD ; Father - dementia ; Mother - stroke
221	Probable bv-FTD	10	Father - vascular dementia at 78
222	Possible FTLD	2	Father - AD, PD, heart disease; Brother - alcohol abuse

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Table 2

A review of the findings from the current study and other studies on subjects found to carry the *C9ORF72* expansion.

	NIH / NINDS *C9ORF72* expansion negative	**NIH / NINDS *C9ORF72* expansion positive**	**DeJesus-Hernandez et al**	**Renton et al**	**Murray et al**	**Gijselnick et al**	**Stewart et al**	**Pearson et al**
Sample	FTD spectrum patients not enriched for FH or MND	FTD spectrum patients not enriched for FH or MND	374 clinical FTLD, out of total of 696 clinical and pathological patients)	75 Finnish FTLD cases	20 expansion+ pathological cases from a brain bank	305 FTLD cases, 137 ALS, 23 with FTD-ALS	231 ALS patients	9 expansion+ cases of ALS-FTD in one family
Expansion prevalence	49	4 8% overall, 3% of sporadic and 15% of familial were expansion +	3% sporadic and 11.7% familial were expansion+	22 (29.3%) expansion+	20	Familial: 86% of FTD-ALS 47% of ALS 16% of FTLD Sporadic: 6% of FTD-ALS 5% of ALS 4% of FTLD	3.6% of sporadic, 27.4% of familal	N.A.
Clinical Diagnoses	4 FTD-ALS 27 probable bv-FTD 9 possible FTLD 6 PPA-PNFA 2 PPA-semantic 4 AD 1 MSA	2 FTD-ALS 2 bv-FTD	For +: 25 bv-FTD 1 language-variant 7 of the 26 also had ALS	For +: 8 PPA-PNFA 16 bv-FTD 8 of the 22 had personal or FH of ALS	8 FTLD 6 ALS 1 FTD-ALS 4 AD 1 Other	Selected for cases of FTLD, ALS, and FTD-ALS	Expansion + cases more frequent bulbar onset and FTD-ALS	5 presented with ALS, 1 with FTD-ALS, and 3 with bv-FTD. 2 developed psychosis, 3 visuo-spatial dysfunction, 4
Mean age of onset	57	58	For +: 56.2	Overall 58.4	61.75	FTLD: 55.3 in carriers, 63.2 years in non-carriers ALS: 54.5 in carriers, 60.4 years in non-carriers	For +: 58.2, for -: 57.4	42.7
+FH of a first-degree relative with dementia or ALS	15/44 (34%)	% (75%)	For +: 37/52 (71%)	Overall 27 (36%)	12/20 (60%)	FTD-ALS: 30% ALS: 10% FTLD: 25%	For +: 73% for -: 15%	N.A.
Evidence of anticipation	N/A	Probands earlier age of onset than parents	Not apparent	Not observed	--	Trend towards younger age of onset between	--	--

	NIH / NINDS *C9ORF72* expansion negative	NIH / NINDS *C9ORF72* expansion positive	DeJesus-Hernandez et al	Renton et al	Murray et al	Gijselnick et al	Stewart et al	Pearson et al
						generations		
FDG-PET	--	1 typical for FTLD 3 c/w FTLD or AD	--	--	--	--	--	--
Pathology	--	1 FTLD-TDP type B with AD pathology	11 – all FTLD-TDP type B	--	FTLD-TDP type B. One case had AD pathology	FTLD-TDP type B, more ubiquitin than TDP-43+ staining	ALS with TDP-ir inclusions and FTLD-TDP type B, more ubiquitin than TDP-43+ staining	FTLD-TDP type B