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GRN and MAPT mutations in two Brazilian dementia research centers

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Abstract

Background—Mutations in *GRN* (progranulin) and *MAPT* (microtubule-associated protein tau) are among the most frequent causes of monogenic Frontotemporal Dementia (FTD), but data on the frequency of those mutations in regions such as Latin America is still lacking.

Objective—We aimed to investigate the frequencies of *GRN* and *MAPT* mutations in FTD cohorts from two Brazilian dementia research centers, from University of Sao Paulo (USP) and Federal University of Minas Gerais (UFMG) medical schools.

Methods—We included 76 probands diagnosed with behavioral variant FTD (n=55), semantic variant Primary Progressive Aphasia (PPA) (n=11), or nonfluent variant PPA (n=10). Twenty-five percent of the cohort had at least one relative affected with FTD.

Results—Mutations in *GRN* were identified in seven probands, and in *MAPT*, in two probands. We identified three novel *GRN* mutations (p.Q130X, p.317Afs*12, and p.K259Afs*23), in patients diagnosed with nvPPA or bvFTD. Plasma progranulin levels were measured and a cutoff value of 70ng/ml was found with 100% sensitivity and specificity to detect null *GRN* mutations.

Conclusions—The frequency of *GRN* mutations was 9.6% and of *MAPT* mutations, was 7.1%. Among familial cases of FTD, the frequency of *GRN* mutations was 31.5%, and of *MAPT* mutations was 10.5%.

Keywords

frontotemporal dementia; primary progressive aphasia; progranulin; tau; genetics

Introduction

An autosomal dominant pattern of inheritance is observed in around 10-15% of Frontotemporal Dementia (FTD) cases.^{1,2} Mutations in more than ten genes are currently known to be causative of monogenic forms of FTD, but the most commonly reported worldwide are found in *GRN* (progranulin), *MAPT* (microtubule-associated protein tau) and *C9orf72* (chromosome 9 open reading frame 72).³ *GRN* mutations cause disease through haploinsufficiency, and progranulin levels are low in plasma and cerebrospinal fluid of mutation carriers.^{4,5}

The frequencies of *MAPT* and *GRN* mutations in FTD cohorts around the world have been published, and results vary significantly. The frequency of *GRN* mutations in Italy, for example, was 15.6% in one study, whereas in Japan, it was 1.6%.^{6,7} The frequencies of *MAPT* mutations in FTD cohorts were 17.9% in a British study, and 4.7% in a French study.^{3,8} World regions such as Latin America and Africa, however, have been largely understudied and data is still scarce on monogenic FTD in those regions.

In this study, we aimed to determine the frequencies of *GRN* and *MAPT* mutations in FTD cohorts seen in two of Brazil's largest dementia research centers, and characterize the demographic, clinical and neuropathological features of mutation carriers. We also used plasma progranulin levels to detect null mutations in *GRN*, and aimed to determine a cutoff value for our population.

Methods

Population

We included patients diagnosed with behavioral variant FTD (bvFTD, n=55), semantic variant Primary Progressive Aphasia (svPPA, n=11), or nonfluent variant PPA (nfvPPA, n=10), with or without motor neuron disease (MND), based on current diagnostic criteria.^{9,10} Four probands had FTD-MND. Patients were seen at the dementia clinics from the University of Sao Paulo (USP cohort) or from the Federal University of Minas Gerais (UFMG cohort), or elsewhere (i.e. private offices) by researchers affiliated with either one of those two institutions. Family history was considered positive for FTD if at least one first degree relative was diagnosed with or had symptoms suggestive of FTD based on information obtained from family members.

Individuals who did not have neurological or psychiatric comorbidities, did not have cognitive complaints or evidence of functional decline, and obtained a score above education-adjusted cutoff values on the mini-mental status examination (MMSE)¹¹ were

selected for the control group. MMSE cutoff scores were as follows: 18 for illiterates, 24 for individuals with one to seven years of schooling, and 26 for individuals with eight or more years of schooling.

Gene sequencing

DNA was extracted from peripheral blood leukocytes using previously described methods.¹² For each PCR reaction, we used 12.5µl of PCR MasterMix (Thermo Scientific®), 4.5µl of autoclaved DEPC-treated water, 2µl of each primer (forward and reverse),^{13,14} and 4µl of DNA (50ng/µl). We used touchdown protocols in an Eppendorf Mastercycler pro® thermocycler (protocols available upon request). Sanger sequencing of exons 1, 9-13 of *MAPT* and 1-12 of *GRN* was performed in ABI 3130XI Genetic Analyzer (Applied Biosystems®). Sequencing data was analyzed with Mutation Surveyor v3.2 (SoftGenetics®).

Plasma progranulin

Blood was centrifuged at 1000×g for 15 minutes on the same day of blood collection, and plasma was stored at -20°C. Plasma progranulin levels were measured using a human Progranulin ELISA kit (Adipogen®), following the manufacturer's instructions. Plates were read at 450nm with Synergy HT (Biotek®) reader.

Statistical analyses

To compare groups, we used Kruskal-Wallis or Wilcoxon-Mann-Whitney tests, depending on the number of groups analyzed. For categorical variables, we used the Chi-square test. Significance was set at $p < 0.01$. Statistical analyses were done with Stata 12.1 (StataCorp LP®).

Neuropathological assessment

Brain was fixed in 10% buffered paraformaldehyde for 14 to 21 days. Brain regions were sampled from the fixed hemispheres, embedded in paraffin and sectioned at 5µm thickness for staining. Areas included frontal pole, anterior orbital gyrus, amygdala at the level of mammillary bodies, middle and inferior frontal gyri, ventral striatum, superior, middle and inferior temporal gyri, insula, basal ganglia at the level of the anterior commissure, superior frontal sulcus, hippocampal formation at the level of lateral geniculate body, sensorimotor cortex, thalamus, angular gyrus, calcarine cortex, cerebellum with dentate nucleus, midbrain, rostral and, caudal pons and rostral medulla. All areas were stained with hematoxylin & eosin and selected sections were immunostained with antibodies against: β-amyloid (4G8, 1:10,000; Signet Pathology Systems, Dedham, MA), phospho-tau (PHF-1, 1:2,000; gift of Peter Davies, NY), TDP-43 (1:500, Proteintech, Chicago, IL) and α-synuclein (EQV-1, 1:10,000; gift of Kenji Ueda, Tokyo, Japan)

This study was approved by the local Ethics Committee and informed consent forms were signed prior to blood draw.

Results

The clinical and demographic features of the cohorts are shown in Table 1. Seventy-six probands were included in this study (62 from the USP cohort and 14 from the UFMG cohort). Nineteen of the probands (25%) had a positive family history for FTD. Only one individual from a family (proband) was included in Table 1.

The control group was composed of sixty individuals who did not carry pathogenic mutations in *GRN* or *MAPT* (Table 1).

GRN mutations

Null *GRN* mutations were identified in seven probands (Table 2). Of those, three are novel (p.Q130X in proband USP 22, p.D317Afs*12 in proband USP 63, and p.K259Afs*23 in proband UFMG 18) and will be presented in detail below.

Proband USP 11 was diagnosed with nfvPPA. Her family history was negative for FTD, but her mother was diagnosed with early-onset Alzheimer's disease (AD) after presenting with memory problems and apathy at age 63. Proband USP 22 was diagnosed with nfvPPA, and her brother developed bvFTD at age 57. Their mother died with a diagnosis of early-onset AD. Proband USP 53 was diagnosed with nfvPPA, but also had severe impairment of semantic memory, and hence could be classified as having mixed PPA.¹⁵ Proband USP 64 was diagnosed with bvFTD. His mother was also diagnosed with bvFTD, with onset of symptoms at age 58 and death at age 69. A first degree cousin developed bvFTD at age 59 and died at age 70, but the cousin's mother died at age 87 without clear signs of cognitive impairment.

The frequency of *GRN* mutations in the USP cohort was 9.6% (6/62), and 7.1% (1/14) in the UFMG cohort. The frequency in the combined cohort was 9.2% (7/76).

GRN p.Q130X

Proband USP 58 started with memory problems and topographic disorientation when she was 50 years old. She was first seen two years later, and scored 19 on the MMSE. Her brain MRI showed global atrophy and FDG-PET showed hypometabolism in the frontal, temporal and parietal regions, as well as in the anterior cingulate area. She was first diagnosed with possible AD, but later developed symptoms consistent with bvFTD, such as loss of empathy, irritability, and hyperphagia. Language problems also appeared, and were characterized by a nonfluent speech, with syntax errors and phonemic paraphasias. Her symptoms worsened over time, and five years after onset of symptoms she was practically mute and was dependent for all basic daily life activities.

Her sister was also diagnosed with bvFTD, and carried the same mutation. At the age of 54, she developed difficulty with planning and organizing, excessive shopping and changes in eating behavior. Her score on the MMSE was 19, one year after onset of symptoms. She later developed delusions and parkinsonism (while in use of an atypical antipsychotic). Her MMSE score was eight, two years after onset of symptoms. Her brain MRI scans are shown in Figure 1. The FLAIR sequence images show progressive atrophy over the course of

disease (the first scan was done in her first year of symptoms), accompanied by white matter hyperintensities that were absent in the first scan and appeared gradually.

The proband's mother died of pulmonary embolism at age 60, without signs of cognitive impairment. Her maternal grandmother had presenile dementia, with onset around the age of 50.

GRN p.D317Afs*12

Proband USP 63 developed behavioral changes characterized by disinhibition, apathy, impulsivity and overspending when he was 52 years old. He was first evaluated at age 54, and scored 29 on the MMSE. The neurological examination did not show signs of parkinsonism or motor changes. His brain MRI showed bilateral frontal atrophy, worse on the right side (Figure 1), and brain SPECT showed bilateral frontal hypoperfusion. He was diagnosed with bvFTD.

His symptoms worsened gradually. Around four years after symptom onset, the neurological examination showed parkinsonism and dystonic posturing of limbs during gait. He died fifteen years after disease onset, at age 67.

The brain was collected at the Brazilian Brain Bank of the University of Sao Paulo Medical School. Unfixed brain weight was 802 grams (Figure 2). Macroscopic examination showed asymmetric global cortical atrophy most significant in the frontal, temporal and parietal lobes. Microscopic examination was performed on the right hemisphere.

Immunohistochemistry against hyperphosphorylated TDP-43 (mouse monoclonal MAb 409/410, 1:1000; Cosmo Bio, Tokyo, Japan) showed the presence of immunoreactive pathology. Neuronal cytoplasmic inclusions and short dystrophic neurites were observed in the frontotemporal cortex predominantly in layer 2. In addition, few neuronal cytoplasmic inclusions were detected in dentate granule cells of hippocampus. The neuropathological findings were consistent with FTLT-DTP (Frontotemporal lobar degeneration with TDP-43 positive inclusions) type A.¹⁶

The proband's mother developed symptoms consistent with nfvPPA when she was 77 years old. Two years later, she developed behavioral symptoms, such as lack of empathy, signs of poor judgment and apathy. Brain MRI showed frontal perisylvian atrophy, slightly worse on the left hemisphere, and periventricular white matter hyperintensities. Around five years after onset of symptoms, parkinsonian signs with gait abnormalities were noticed. She is currently alive, at age 89, but is mute and unable to stand or walk.

GRN p.K259Afs*23

Proband UFMG 18 is a male patient who presented with word-finding difficulties at age 69. He had 16 years of schooling. On neurological examination, his MMSE score was 26/30, with mild impairment in confrontation naming and category fluency. His gait was normal, and no motor or sensory changes were observed. The brain MRI did not show any abnormality at the time. Slow deterioration of naming was recorded, with increasing interference in his professional activities. Apathetic behavior emerged one year after first examination, followed by disinhibition six months later. Eating changes (preference for

sweets) were also reported. This clinical picture worsened progressively. MRI revealed marked atrophy of the anterior and medial parts of the left temporal lobe (Figure 1). He was diagnosed with bvFTD. Three years after first examination he scored 11/30 on the MMSE, with marked reduction in verbal fluency. No motor or sensory changes were observed.

His family history was positive for bvFTD. His father died with bvFTD at age 77, five years after onset of symptoms. Two of his siblings were also diagnosed with bvFTD. One of his two brothers developed symptoms at age 66, and died at age 70 and his sister's symptoms began at age 64. She died when she was 75 years old. The proband's cousin was also diagnosed with bvFTD and carries the same mutation. At age 70, he presented with a twelve-month history of memory deficits, word-finding difficulties and logorrheic speech. Other medical problems included obstructive sleep apnea, one episode of syncope of unknown etiology and Paget's disease of bone. On neurological examination, his MMSE score was 27 (loss of three points on word recall), with mild impairment in confrontation naming and category fluency (semantic verbal fluency animal category = 11, phonemic verbal fluency [FAS]=31, and Boston naming test 39/60). Gait was normal without clinical signs of cerebrovascular disease, parkinsonism or motor neuron disease. Brain MRI showed white matter hyperintensities which were interpreted to be due to microangiopathy. Deterioration of language and apathy was noticed in the following 12 months with interference with his daily activities (MMSE score 24). Three years later he presented with marked clinical deterioration especially on verbal fluency (MMSE 22, semantic verbal fluency animal category = 6, phonemic verbal fluency [FAS]=19), stereotypical speech without any change in neurological examination. MRI revealed marked atrophy of the anterior and medial parts of the left temporal lobe.

MAPT mutations

Two probands had mutations in *MAPT* (Table 2). Proband USP 17 was initially diagnosed with bvFTD, but later developed a PSP-like (progressive supranuclear palsy) phenotype. Four of his siblings had died after developing similar behavioral and motor symptoms (suggestive of PSP). Proband UFMG 23 was diagnosed with bvFTD, and had a deceased sister who had developed dementia with frontotemporal features.

The frequency of *MAPT* mutations in the USP cohort was 1.6% (1/62), and in the UFMG cohort was 7.1% (1/14). The frequency in the combined cohorts was 2.6% (2/76).

Plasma progranulin levels

The means, standard deviations and range of plasma progranulin levels are shown in Figure 3. We included plasma samples from the control group (n=60), USP FTD cohort (55 GRN- probands, 4 GRN+ probands and 3 symptomatic GRN+ relatives) and two individuals from the UFMG FTD cohort (n=2, proband UFMG 18 and a symptomatic first-degree cousin who carried the same mutation). Plasma samples from probands USP 58 and USP 63 were not available, but we obtained plasma from the sister of proband USP 58 and mother of proband USP 63 (as well as from the brother of proband USP 22).

Plasma progranulin levels were significantly lower in the FTD group with null GRN mutations (FTD GRN+) than in the FTD group without GRN mutations (FTD GRN-),

$p < 0.0001$) or in the control group ($p < 0.0001$). Nonparametric ROC analysis (FTD GRN+ vs. FTD GRN- and control) showed a cutoff value of 70ng/ml indicated null *GRN* mutations with 100% sensitivity and specificity.

Plasma progranulin levels were also lower in the FTD GRN- group, in comparison to the control group ($p < 0.0001$). The FTD GRN- and control groups were matched for age at blood draw and sex ($p = 0.96$ and $p = 0.127$, respectively).

Discussion

The frequency of *GRN* mutations in this Brazilian FTD cohort was 9.6%, and among familial FTD cases, the frequency was 31.5%. *MAPT* mutations were found in 7.1% of FTD cases and 10.5% of familial FTD cases. We did not find *GRN* or *MAPT* mutations in sporadic FTD, but one proband's (USP 11) family history only included a mother diagnosed with early-onset AD (and therefore was not considered as having familial FTD). Three of the *GRN* mutations (p.Q130X, p.D317Afs*12, and p.K259Afs*23) were not previously reported in FTD, highlighting the importance of studying genetics in different populations. This is the first study on the frequency of *GRN* and *MAPT* mutations in South American FTD cohorts.

The frequency of *GRN* mutations in this cohort is within the range reported by North American and European studies (3-15%),^{3,6,8,17-20} but higher than reported by Asian groups (0-1.6%).^{7,21,22} Among familial FTD cases, the frequency of *GRN* mutations is higher than reported by other groups, such as studies conducted in Northern Italy (24.8%), United Kingdom (20%) or France (14%).^{3,6,8} Similarly, the frequency of *MAPT* mutations in our cohort is within the range published by groups from the USA, Japan, and European countries (4.7-17.9%).^{3,7,8,17-19} *MAPT* mutations were absent in Korean and Indian cohorts.^{21,22} A subset of the USP FTD cohort (n=47) were screened for *TARDBP* mutations, and a p.I383V mutation was identified in a proband diagnosed with svPPA.²³

The *GRN* p.Q130X mutation is a nonsense mutation found in two sisters diagnosed with bvFTD, one of which had low levels of plasma progranulin (25ng/ml). Although it has not been reported in patients with FTD, the mutation was identified in a tissue sample of a 63-year-old man with large intestine carcinoma in the COSMIC catalog of somatic mutations in cancer (COSM187683).²⁴ No data is available on cognitive symptoms.

The *GRN* p.D317Afs*12 mutation was identified in a proband diagnosed with bvFTD, who developed symptoms at age 52 and died 15 years later. The neuropathological assessment revealed changes compatible with FTLT-TDP type A, which is the subtype typically associated with *GRN* mutations.¹⁶ The proband's mother was also tested for *GRN* mutations, and carries the same mutation; and her plasma progranulin level was low (48.4ng/ml). Further corroborating the pathogenicity of this mutation, the SIFT indel algorithm predicted nonsense-mediated messenger RNA decay caused by the *GRN* g.11444-11445delAC mutation.²⁵

The *GRN* p.K259Afs*23 mutation was found in a proband diagnosed with bvFTD. The SIFT indel algorithm also predicted nonsense-mediated messenger RNA decay caused by g.

10981-10984delAAGT.²⁵ Sequencing *GRN* in a first-degree cousin of the proband, diagnosed with bvFTD, revealed the same mutation, indicating segregation with disease. Moreover, as expected for a null *GRN* mutation, plasma progranulin levels were low in both patients (17.1ng/ml in the proband, and 60.2ng/ml in his cousin), which provides further evidence of pathogenicity.

The remaining *GRN* null mutations (*GRN* p.Q300X, p.V200Gfs*18, p.Q257Pfs*27 and p.S301Cfs*61) have been reported elsewhere, in British (p.Q300X), French (p.V200Gfs*18), Swedish (p.V200Gfs*18), Canadian (p.V200Gfs*18) and Portuguese (p.Q257Pfs*27 and p.S301Cfs*61) FTD cohorts.^{8,20,26-31} Of note, the Canadian patients with the *GRN* p.V200fs*18 mutation were two sisters of Chinese ancestry diagnosed with corticobasal syndrome.²⁹ The observation that two of the mutations we found in this study (*GRN* p.Q257Pfs*27 and *GRN* p.S301Cfs*61) have only been reported in Portuguese FTD cohorts is interesting, but not entirely unexpected, considering Brazil's history as a Portuguese colony.^{26,31} The maternal grandfather of proband USP 64 was born in Portugal and emigrated to Brazil in the beginning of the 20th century.

The clinical presentation of *GRN*-associated disease was highly variable, which is in line with previous reports.^{27,32} Three of the probands were diagnosed with nvfPPA, whereas the other four probands were diagnosed with bvFTD. *GRN* mutations were found in 7.2% (4/55) of bvFTD cases and 30% (3/10) of nvfPPA cases. No *GRN* mutations were identified in svPPA or FTD-MND cases. There was also significant intrafamilial heterogeneity. Proband USP 63, for example, developed symptoms at an age twenty five years earlier than his mother. Similarly, Rademakers et al. observed families in which age at onset or death occurred 10-20 years later in a parent than in the proband.³²

Neuroimaging studies have reported white matter abnormalities in 20-40% of *GRN* mutation carriers.^{30,31} We observed periventricular white matter hyperintensities in four of the six mutation carriers from whom FLAIR/T2-weighted MRI scans were available. As shown in Figure 2A, however, white matter changes may be absent in the early stages of disease and only appear in areas of maximal atrophy as the disease progresses.

In this study, we found that plasma progranulin levels ≥ 70 ng/ml identified null *GRN* mutations with 100% sensitivity and specificity. This cutoff value is similar to the one reported by Ghidoni et al. (61.55ng/ml), but lower than the value suggested by Finch et al. (112ng/ml).^{4,5} Upon reviewing the data reported by groups that also used the Adipogen[®] ELISA kit to measure progranulin levels in plasma, we found significant variability across studies. In controls, the mean plasma progranulin level varied between 169 and 228 ng/ml, and the measurements ranged between 61.8 and 473.4ng/ml.^{4,5,3,35} Among null *GRN* mutation carriers, plasma progranulin levels ranged from 10.9 to 107.8ng/ml.^{4,5,34} Of note, the higher end of this range is close to the mean value we found in the FTD group without *GRN* mutations (109ng/ml). The reasons for these discrepancies are not clear, but are likely due to different demographic characteristics of the cohorts (age and gender are known to affect plasma progranulin levels), as well as other genetic factors that influence progranulin expression.³⁶ It should be noted that the Brazilian population has significant genetic admixture and it is possible that the frequencies of genetic polymorphisms that influence

plasma progranulin levels differ in our population, and this could explain at least in part our findings (even though this has not been formally tested).

Both *MAPT* mutations (p.N279K and IVS10+16C>T) have already been reported, and were found in 2 probands with bvFTD (3.6% of bvFTD cases). Mutations in *MAPT* were not identified in probands with nvPPA, svPPA or FTD-MND. The *MAPT* p.N279K mutation was identified in a patient (USP 17) who first developed behavioral symptoms suggestive of bvFTD, but later showed axial-predominant parkinsonian features and eye movement abnormalities consistent with a diagnosis of PSP. The PSP phenotype is the most commonly reported presentation of this mutation.^{7,36} The p.N279K mutation was reported in a large north American kindred diagnosed with pallido-ponto-nigral degeneration, and also in French, Italian and Japanese families.^{7,37,38} The IVS10+16C>T is one of the most common *MAPT* mutations reported worldwide, and was identified in at least 27 families.³⁹ Patients with this mutation are more often diagnosed with bvFTD (such as the proband UFMG 23), but an AD-like phenotype has also been reported.⁴⁰

In conclusion, the frequencies of *GRN* and *MAPT* mutations in this Brazilian FTD cohort were 9.6% and 7.1%, respectively. *GRN* mutations were particularly frequent among familial FTD cases, in a frequency (31.5%) that is higher than found by other groups. Three novel null *GRN* mutations were identified (p.Q130X, p.D317Afs*12, and p.K259Afs*23), and were reported with solid evidence of pathogenicity. A plasma progranulin cutoff value of 70ng/ml was associated with 100% sensitivity and specificity to detect null *GRN* mutations, though a study with a larger sample of Brazilian FTD patients and controls would be necessary to corroborate this finding.

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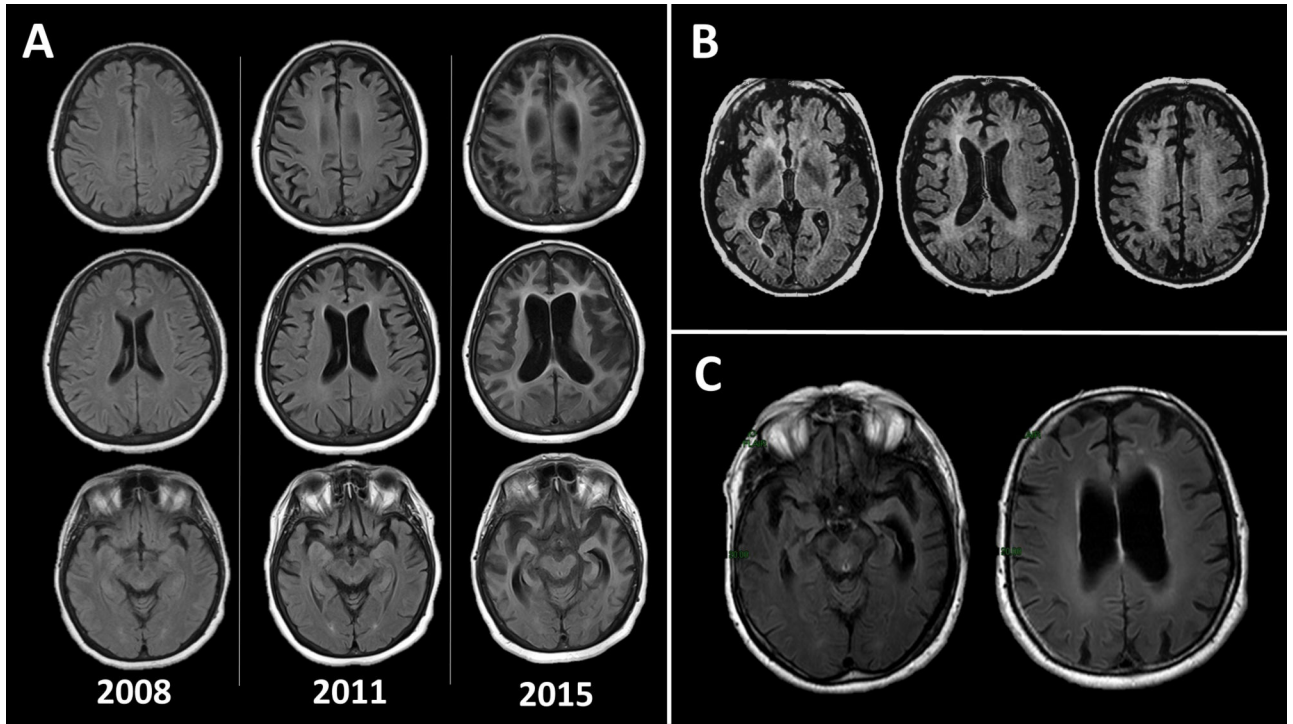


Figure 1.

Neuroimaging of probands

Brain MRI scans (A) Proband USP 58's sister. Axial FLAIR images. White matter hyperintensities appear as atrophy in frontal, temporal and parietal regions progress. (B)

Axial FLAIR images from proband USP 64 show global cortical atrophy with

periventricular white matter hyperintensities. (C) Axial FLAIR images from Proband UFMG 18 show left anteromedial temporal lobe atrophy and subtle white matter hyperintensities.

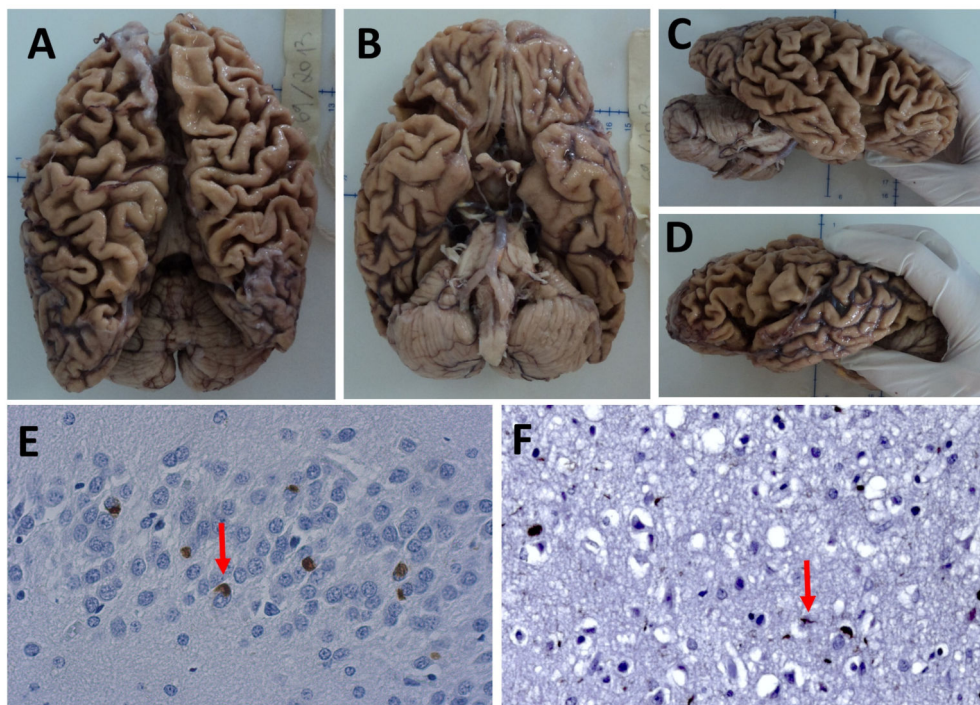
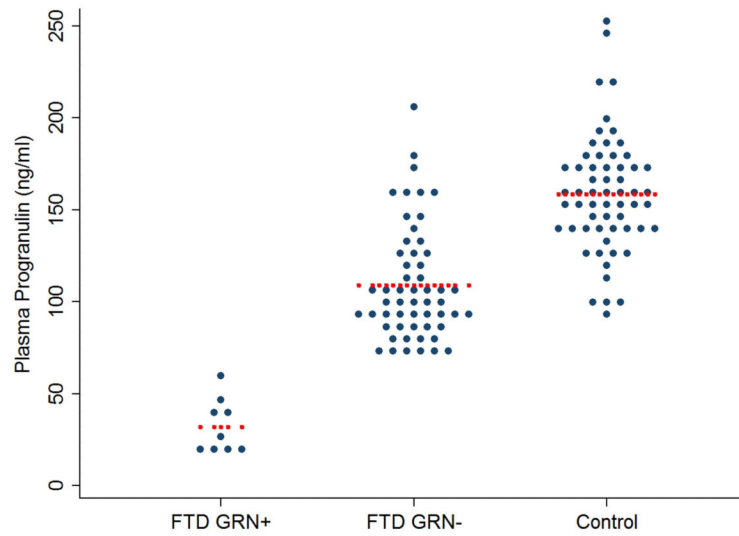


Figure 2.

Neuropathological findings in proband USP 63

Macroscopy (A-D): global cortical atrophy, predominantly in the frontal, temporal and parietal lobes. Atrophy is slightly more significant on the right hemisphere. Views: dorsal (A); ventral (B); lateral of right hemisphere (C); lateral of left hemisphere (D). Microscopy (E-F, 400x): Immunohistochemistry with antibodies against hyperphosphorylated TDP-43 showing neuronal cytoplasmic inclusions (red arrow) in the granule cell layer of the dentate gyrus (E) and short dystrophic neurites (red arrow) in the entorhinal cortex (F).



Groups	N	Plasma progranulin level (ng/ml)
FTD GRN+	9	31.7±15.4 (17.1 – 60.2)
FTD GRN-	55	109.0±30.7 (70.2 – 206.3)
Control	60	158.4±32.0 (94.8 – 249.9)

Figure 3.

Dot plot of plasma progranulin levels.

FTD: frontotemporal dementia; GRN+: with null GRN mutations; GRN-: without null GRN mutations. Values shown as mean ± standard deviation (range) in ng/ml. Red dotted lines indicate the means.

Table 1

Demographic and clinical characteristics of the USP and UFMG cohorts, and control group

	N	Sex	Age at onset [#]	MND	FH+ FTD (%)
Entire Cohort					
bvFTD	55	28M:27F	54.1±10.0 (35-73)	3	13 (23.6%)
nvPPA	10	3M:7F	59.5±6.1 (48-71)	0	4 (40%)
svPPA	11	7M:4F	64.3±6.6 (50-71)	1	2 (18.1%)
FTD	76	38M:38F	56.1±9.7 (35-73)	4	19 (25%)
USP Cohort					
bvFTD	44	22M:22F	52.1±9.7 (35-73)	3	10 (22.7%)
nvPPA	9	2M:7F	63.7±6.9 (50-71)	0	3 (33.3%)
svPPA	9	6M:3F	59.8±6.4 (48-71)	1	2 (22.2%)
FTD	62	30M:32F	54.8±9.8 (35-73)	4	15 (24.1%)
UFMG Cohort					
bvFTD	11	6M:5F	62.4±6.6 (49-70)	0	3 (27.2%)
nvPPA	1	1M:0F	(69)	0	1 (100%)
svPPA	2	1M:1F	(56-67)	0	0
FTD	14	8M:6F	62.4±6.5 (49-70)	0	4 (28.5%)
Control group	N	Sex	Age at blood draw [#]	MND	FH+ FTD (%)
Control	60	21M:39F	60.8±8.5 years (40-84)	NA	NA

Legend: FTD: frontotemporal dementia; bvFTD: behavioral variant FTD; PPA: primary progressive aphasia; nvPPA: nonfluent variant PPA; svPPA: semantic variant PPA; M: male; F: female; MND: motor neuron disease; FH+: positive family history.

[#]: shown as mean ± standard deviation (range) in years.

Table 2*GRN* and *MAPT* mutations

Proband	Change in genomic sequence [#]	Change in protein sequence [*]	Clinical phenotype	Age at onset of symptoms (years), Sex
<i>GRN</i> mutations				
USP 11	g.10976_10977dupCC	p.Q257Pfs*27	nvPPA	60, F
USP 22	g.11303C>T	p.Q300X	nvPPA	63, F
USP 53	g.10679G>C	p.V200Gfs*18	nvPPA/ mixed PPA	65, M
USP 58	g.10144C>T	p.Q130X	bvFTD	48, F
USP 63	g.11444-11445delAC	p.D317Afs*12	bvFTD	52, M
USP 64	g.11307-11308dupGT	p.S301Cfs*61	bvFTD	43, M
UFMG 18	g.10981-10984delAAGT	p.K259Afs*23	nvPPA	69, M
<i>MAPT</i> mutations				
USP 17	g.120904T>G	p.N279K	bvFTD/PSP	45, M
UFMG 23	g.120998C>T (IVS10+16C>T)	-	bvFTD	63, F

[#]: Legend: relative to nt1 in NG_007886.1 (*GRN*) or NG_007388.1 (*MAPT*);

^{*}: numbering based on NP_002078.1 (*GRN*) and NP_002078.1 (*MAPT*). M= male; F= female.