A pathogenic progranulin mutation and C9orf72 repeat expansion in a family with frontotemporal dementia

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Aims: Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative disease and is the second most common form of young onset dementia after Alzheimer’s disease (AD). An autosomal dominant pattern of inheritance is present in around 25–50% of FTLD cases indicating a strong genetic component. Major pathogenic mutations of FTLD have been demonstrated independently in the progranulin (GRN) gene and the C9orf72 hexanucleotide expansion repeat. In this study we present a family that have been identified as carrying both a GRN Cys31fs mutation and the C9orf72 hexanucleotide expansion repeat.

Methods: In the present study we describe the clinical and genetic details of family members and pathological features of two family members that have come to post-mortem.

Results: The mean age at disease onset was 57 years (48–61 years) and mean duration 4 years (2–7 years). The most common presenting syndrome was behavioural variant frontotemporal dementia. Brain imaging from available cases showed a symmetrical pattern of atrophy particularly affecting the frontal and temporal lobes. Pathologically two cases were classified as FTLD-TDP type A with TDP-43 positive inclusions, with additional p62-positive ‘star-like’ inclusions found in the hippocampal formation and cerebellum.

Conclusions: The type and distribution of the pathological lesions in these two cases were in keeping with FTLD cases carrying only the C9orf72 hexanucleotide repeat. However the driving force of the pathological process may be either pathogenic mutation or a combination of both converging on a singular mechanism.

Keywords: C9orf72, FTLD, progranulin, TDP-43

Introduction

Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative disease and is the second most common form of dementia with presenile onset after Alzheimer’s disease (AD). Clinically, there are three major syndromes, behavioural variant frontotemporal dementia (bvFTD), semantic dementia (SD) and progressive non-fluent aphasia (PNFA), whilst pathologically, the majority of cases have inclusions containing either tau (FTLD-tau) or TDP-43 (FTLD-TDP). A family history of FTLD, often showing an autosomal dominant pattern of inheritance is present in around 25–50% of cases indicating a strong genetic component [1,2]. Major genetic causes of FTLD include mutations in the MAPT [3] and progranulin (GRN) genes [4] both located on chromosome 17. Recently, two independent studies identified a GGGGCC
hexanucleotide expansion in the chromosome 9 open reading frame 72 (C9orf72) gene as a further common genetic cause of FTLD [5,6]. The clinical phenotype of individuals with a GRN mutation is variable with patients presenting with bvFTD, PNF A or in some cases, a corticobasal syndrome [7]. The first GRN mutation associated with FTLD was identified in 2006. Since then over 70 different GRN mutations have been reported in more than 230 families worldwide, which accounts for 5–20% of cases of familial FTD and 1–5% of sporadic cases [8]. Most mutations are predicted to result in premature termination codons and it has been shown that the mutant mRNA is rapidly degraded through the process of nonsense mediated decay, resulting in a functional null allele [4]. All cases with known GRN mutations are associated with underlying FTLD-TDP type A pathology, characterized by neuronal cytoplasmic inclusions (NCIs), occasional neuronal intranuclear inclusions (NIIs) and short neurites.

C9orf72 expansions are associated with a heterogeneous clinical presentation and are highly variable between and within family members [9]. Between 7% and 12% of all FTD cohorts are found to have the mutation [9–11]. Patients can present with FTD, ALS or mimic several other neurodegenerative disease syndromes [12]. The FTD subtype is most often bvFTD with PNF A being observed occasionally. ALS typically shows early involvement of both upper and lower motor neurones and bulbar presentation is common [13–18]. The neuropathology associated with the C9orf72 expansion repeat combines both FTLD-TDP and ALS. Previous mutations associated with FTD, have fallen neatly into one of the pathologically determined subtypes; GRN with FTLD-TDP type A and VCP with FTLD-TDP type D. However, the C9orf72 expansion repeat crosses the pathological boundaries and has been shown to be associated with two FTLD-TDP subtypes; type A and B [9]. Cases with this expansion also show unique pathology in addition to the TDP-43 inclusions with TDP-43 negative, p62-positive NCIs found predominantly in the hippocampal formation and cerebellar granule cell layer [9,19].

In the present study we present a family that have been identified as carrying both a GRN Cys31fs (g.90_91insCTGC) pathogenic mutation in addition to the C9orf72 hexanucleotide expansion repeat. We describe the clinical, genetic and pathological features of two of the family members that have now come to post-mortem. We compare the clinical and pathological findings with FTLD cases affected by either a GRN or a C9orf72 hexanucleotide expansion and discuss the relevance of both mutations, speculating which may be driving the pathogenic mechanism.

Material and methods

Cases

The two reported cases donated their brains to the Queen Square Brain Bank but had clinically been seen in the Dementia Research Centre (UCL), they underwent a standard clinical history, neurological examination and cognitive assessment. Ethical approval for the study was obtained from the National Hospital for Neurology and Neurosurgery Local Research Ethics Committee.

Genetics

DNA extraction. Rs3849942 genotyping, hexanucleotide repeat number assessment, DNA sequencing and Southern blotting were carried out as described previously [5,6,12].

Assessment of brain volume

We assessed this in a formal analysis of the volume difference between the right and left hemispheres in a group of 15 patients with C9orf72 expansions [10 men, 5 women, mean (standard deviation) age 59.5 (7.8)], 10 patients with GRN mutations [6 men, 4 women, 61.0 (7.0)] and 15 cognitively normal controls [10 men, 5 women, 57.7 (5.3)] using the following methods [20]: a whole-brain region was created by segmenting the brain using a semi-automated technique in the MIDAS software package [21]. Scans and associated whole-brain regions were then transformed into standard space by registration to the Montreal Neurological Institute (MNI) Template. The left and right hemispheric regions were defined using the MNI average brain which was split by dividing the whole volume along a line coincident with the interhemispheric fissure. An intersection of each individual’s brain region and the hemispheric regions defined on the MNI template was generated to provide a measure of brain volume in left and right hemispheres, with the difference between the two hemispheres calculated by subtracting one from the other.

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Immunohistochemistry

Brains were routinely dissected and blocks removed according to the Queen Square Brain Bank protocol. Seven micron thick tissue sections were immunostained using commercially available antibodies to the following proteins: TDP-43 (Abnova, Taipei City, Taiwan; 1:800); p62 (BD Transduction Labs, Oxford, UK; 1:200); ubiquitin (Dako, Ely, UK; 1:200); ubiquilin2 (Abnova, Taipei City, Taiwan; 1:1000); α-synuclein (Vector, Peterborough, UK; 1:50); tau (AT8 clone; Autogen Bioclear, Wiltshire, UK; 1:600); CD68 (Dako, Ely, UK; 1:150), GFAP (Dako, Ely, UK; 1:1000) as previously described [22]. Briefly, immunohistochemistry for all antibodies required pressure cooker pre-treatment in citrate buffer pH 6.0. Endogenous peroxidase activity was blocked with 0.3% H2O2 in methanol and non-specific binding with 10% dried milk solution. Tissue sections were incubated with the primary antibodies, followed by biotinylated anti-mouse IgG (1:200, 30 minutes; DAKO) and ABC complex (30 minutes; DAKO). Colour was developed with di-aminobenzidine/H2O2. Double-label immunofluorescence was carried out on the hippocampus and cerebellum using the anti-p62 antibody in combination with anti-TDP-43 or anti-ubiquilin2 antibodies. After appropriate pre-treatment tissue sections were incubated with anti-p62 antibody as described above, followed by a biotinylated secondary antibody (Dako, 1:200), ABC and developed using the TSA Rhodamine kit (Roche). Sections were then incubated with either the anti-TDP-43 or anti-ubiquilin2 antibody followed by Alexa Fluor 488 (Invitrogen, Paisley, UK; 1:300) for one hour at room temperature. 4′,6-diamidino-2-phenylindol (DAPI) was used for nuclear counterstaining. Appropriate controls were used to confirm that no cross reactivity existed between the visualization of the two antibodies. Sections were viewed with a Leica DM5500B fluorescence microscope using 3D deconvolution post-processing.

Assessment of p62 and TDP-43 pathology

The extent and severity of p62 and TDP-43 positive pathology was evaluated in cases with only a GRN mutation or C9orf72 expansion repeat along with the two cases carrying the double mutations (cases 2.1 and 2.2). A five-tiered semi-quantitative grading scale was used in which the pathological lesions were scored as ‘0’ describing the absence of p62-positive NCI{s and NIIs, score ‘+’ corresponded to 1–5 inclusions present in an average of at least five microscopic fields using a ×20 objective, score ‘++’ was given when the number of lesions was 6–10 while score ‘++++’ was given when the number of inclusions was between 11 and 20. Score ‘++++’ corresponded to greater than 20 lesions.

Results

Clinical history

The DRC 240 family are from the south of the UK and have an autosomal dominant history of speech, cognitive and gait impairment (Figure 1). Little is known about previous generations but case 1.2 was said to have dementia with impaired speech and gait from the age of 61, dying 2 years later whilst case 1.3 also had a young onset dementia with onset at 60 and disease duration of 4 years.

Case 2.1 This 62-year-old woman presented with a 3-year history of speech, gait, behavioural and cognitive impairment. Her initial problem was of impaired articulation which steadily deteriorated with subsequent development of an unsteady gait. Over the same time period she had developed a change in her personality, becoming less extrovert than previously and less interested in doing her normal hobbies. In the year before presentation she also developed impairment of her language, spelling and arithmetical skills as well as dysphagia. She had coeliac disease but had been otherwise well previously. On examination her Mini Mental State Examination (MMSE) was 27/30. She had a bulbar dysarthria with decreased palatal elevation and orofacial apraxia. She had mild generalized wasting in the limbs but no fasciculation’s. Power was mildly decreased in all muscle groups. Reflexes were brisk throughout with downgoing plantars. She had bilateral
dysdiadochokinesis and gait ataxia. Neuropsychometry showed a verbal IQ of 84 and performance IQ of 86. Episodic memory was impaired with difficulties on tasks of naming, spelling, calculation and limb praxis. There was evidence of executive dysfunction but visuoperceptual skills were intact. EMG showed evidence of mild chronic denervation in tibialis anterior bilaterally but was otherwise normal. MRI brain showed relatively symmetrical generalized cortical atrophy with a frontotemporal predominance as well as mild cerebellar atrophy (Figure 2). A diagnosis of probable FTD-MND was made at this time although the features of ataxia were felt to be unusual. She was not seen again and died 4 years later.

Case 2.2 This 48-year-old woman presented with a 2-year history of speech, gait and cognitive impairment. She had coeliac disease but had been otherwise well previously. A CT scan showed mild symmetrical generalized atrophy. She died 2 years later.

Genetics

GRN gene sequencing of DNA from both frozen brain and peripheral lymphocytes demonstrated the frameshift mutation Cys31fs (g.90_91insCTGC) in exon 2 that results in a null allele reported to be pathogenic [23].

rpPCR assessment of C9orf72 hexonucleotide number demonstrated a minimum of 67 repeats in DNA from both patients on one allele and 2 repeats on the other allele. The presence of a large expansion was confirmed by Southern blotting (Figure 3) which revealed a maximum repeat number in the DNA from brain from patient 1 (PDG1083) as 4153 repeats, minimum 1205 (modals measured at 3822, 2500 and 2050 repeats), and in DNA from peripheral lymphocytes from case 2.1 (PDG 3296) a maximum of 3316 was measured and minimum of 812 (modal 2780). Genotyping of rs3849942 demonstrated heterozygosity at this polymorphic site for both patients.
Pathology

Macroscopic observations  Cases 2.1 and 2.2 came to post-mortem. The unfixed brain of case 2.1 weighed 1000 g, the left-half brain weighed 452 g after fixation in 10% buffered formaldehyde. There was a significant degree of frontal atrophy, which was also apparent over the medial surface. Coronal slices revealed the left lateral ventricle to be significantly dilated and focal thinning of most of the frontal cortex. There was severe reduction in bulk of the frontal white matter while the white matter of the temporal lobe was relatively better preserved. The lateral aspect of the putamen showed significant grey discolouration, although its bulk was not apparently reduced. However, the size of the caudate was reduced and it had a flattened rather than convex outline towards the left frontal horn. The thalamus was significantly reduced in bulk, whereas the globus pallidus, subthalamic nucleus, amygdala and hippocampus were of normal size. The substantia nigra showed severe pallor, whereas the locus coeruleus was well pigmented. The proportions of the pontine tegmentum and base were preserved and, in particular, the transverse pontine fibres were clearly visible. The medulla, the cerebellar cortex, white matter and dentate nucleus were also of normal appearance. In case 2.2 the right fixed half brain weighed 516 g. Some of the gyri in the frontoparietal region were narrowed and the intervening sulci widened. Coronal slices showed that the right lateral ventricle was moderately enlarged. The cortical ribbon had a blotchy appearance and the border between the cortex and white matter in many places appeared blurred. The hippocampus was macroscopically unremarkable. The substantia nigra and the locus coeruleus were pale. The cerebellum showed no macroscopic abnormality.

Microscopic observations  Case 2.1 and 2.2 were both previously diagnosed pathologically as FTLD-TDP type A with GRN mutation. Both cases demonstrated TDP-43 NCIs predominantly in layer 2 of the cortex. Numerous small neurites and occasional NIs were also seen (Figure S1). However, with the identification of p62-positive inclusions in the hippocampus and cerebellum the cases were screened for the C9orf72 expansion repeat. A detailed pathological review of both cases was undertaken due to the identification of the second pathogenic C9orf72 mutation.

Cerebral cortex

Histological slides of the frontal cortex from both cases showed a significant degree of superficial spongiosis, moderate degree of neuronal loss and astrogliosis in the grey matter. TDP-43 immunohistochemistry demonstrated moderate numbers of neurites and TDP-43-positive NCIs. The parietal cortex also showed superficial spongiosis and the presence of TDP-43 positive NCIs were observed. The temporal and occipital cortices also contained the occasional TDP-43 NCI to a lesser extent. p62 immunohistochemistry highlighted the TDP-43 positive inclusions, although a minority of NCIs exhibited a ‘star-like’ shape, which were negative for TDP-43.

Hippocampal formation

In case 2.1 neurofibrillary tangles (NFTs) and neuropil threads (NTs) were found in the hippocampus, entorhinal and transentorhinal cortices. TDP-43 positive NCIs were observed in the granule cells of the dentate fascia but these were outnumbered by numerous p62-positive ‘star-like’ inclusions (Figure 4). The majority of the p62-positive inclusions were also immunostained with ubiquitin2 (Figure 4), although there were still inclusions that were only positively stained with p62. The p62-positive ‘star-like’ inclusions were also found in the CA4 subregion, which was negative for TDP-43. Case 2.2
Double pathogenic mutation in FTLD-TDP

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showed a similar distribution of TDP-43 and p62 inclusions as that observed in cases 2.1; however no additional pathology was evident.

Subcortical grey nuclei

The putamen and caudate showed gliosis in case 2.1 confirmed with GFAP immunohistochemistry, where the dorsal regions of these nuclei were more affected than the ventral parts. The subthalamic nucleus was well populated with neurones. Immunostaining for TDP-43 showed numerous neurites in the striatum, some of which had a swollen appearance. NIs and NCIs, both skein-like and compact were also found. Subcortical grey nuclei were not available for analysis from case 2.2.

Brainstem

Case 2.1 demonstrated a significant loss of pigmented neurones together with pigment incontinence and gliosis in the substantia nigra, whereas case 2.2 showed only rare extraneuronal pigment. There were no NFTs or Lewy bodies observed in the nigra or other midbrain nuclei in either case. The locus coeruleus was well populated with neurones as were the griseum pontis and other pontine nuclei. The inferior olive showed mild loss of neurones with sparse TDP-43-positive irregular inclusions. No TDP-43 or p62-positive inclusions are identified in the XIIth nerve nucleus in case 2.1.

Cerebellum

The cerebellar cortex in both cases 2.1 and 2.2 showed a moderate degree of Purkinje cell loss and Bergmann gliosis, while the cerebellar dentate nucleus showed a slight depletion of neurones. No TDP-43-positive inclusions were found in any of the cerebellar subregions. However, frequent p62-positive inclusions were found in the cerebellar granule cell layer (Figure 4).

Additional pathologies

Case 2.1 also demonstrated pathological ageing (Braak and Braak stage II NFT pathology) and cerebrovascular (small vessel) disease, although no Aβ or alpha-synuclein pathology was observed. Whereas in case 2.2 no additional Aβ, tau or alpha-synuclein pathology was observed.

Clinical comparison with C9orf72 and GRN only cases

The main clinical features in the family are of progressive speech, gait and cognitive impairment. In the case for which most information is known (2.1), a diagnosis of FTD-MND was made with prominent bulbar features. This would be most consistent with an expansion in C9orf72 although there are rare cases of FTD-MND in association with GRN mutations. Interestingly, case 2.1 had cerebellar features clinically, which are not seen in GRN mutations, but have been described in some cases of C9orf72 (Table 1). Case 2.1 had impaired executive function, episodic memory and dominant parietal lobe function – this might be seen in either GRN or C9orf72, with early episodic memory impairment perhaps more suggestive of C9orf72, and early parietal lobe involvement more suggestive of GRN, although none of these cognitive features are particularly specific. The brain imaging showed a relatively symmetrical pattern of atrophy (Figure 2), which would be much more in keeping with an expansion in C9orf72 in comparison to GRN mutations where atrophy is asymmetrical. We assessed this in a formal analysis of the volume difference between the right and left hemispheres. In case 2.1, the volume difference between the right and left hemispheres was 10 ml which is not significantly different to a series of fifteen patients with C9orf72 expansions where the mean (standard deviation) right-left hemisphere volume difference was 13 ml (9 ml), or a group of fifteen age and gender matched cognitively normal controls where the volume difference was 5 ml (5 ml). These were all significantly different to the asymmetrical GRN mutation series (10 patients) where the volume difference was 66 ml (30 ml).

Pathological comparison with C9orf72 and GRN only cases

The microscopic pathology observed in cases 2.1 and 2.2 was compared with that seen in 3 heterozygous C9orf72 cases and 3 heterozygous cases carrying only the frameshift Cys31fs mutation in exon 2 of GRN gene. We compared representative areas of the frontal and temporal cortices along with hippocampal subregions and cerebellum (Table 2). In all C9orf72 mutation cases along with the TDP-43 positive inclusions, additional p62-positive ‘star-like’ inclusions were found in the granule cells of the
### Table 1. Demographic and clinical data from GRN/C9orf72 cases compared to GRN and C9orf72 cases

| Demographics | C9orf72 Case 1 | C9orf72 Case 2 | C9orf72 Case 3 | C9orf72/GRN Case 2.1 | C9orf72/GRN Case 2.2 | GRN Case 1 | GRN Case 2 | GRN Case 3 |
|--------------|----------------|----------------|----------------|------------------------|----------------------|------------|------------|------------|
| **Gender**   | Female         | Male           | Male           | Female                 | Male                 | Male       | Male       | Male       |
| **Age at onset** | 68            | 62             | 51             | 59                     | 46                   | 53         | 56         | 66         |
| **Age at death** | 75            | 72             | 57             | 66                     | 49                   | 61         | 64         | 72         |

#### Clinical features

| Main syndrome | FTD-MND | bvFTD | FTD-MND | FTD-MND | bvFTD | bvFTD |
|---------------|---------|-------|---------|---------|-------|-------|

#### Neuropsychiatric and behavioural features

| Disinhibition | + | − | + | − | + | − | + | − | + |
|---------------|---|---|---|---|---|---|---|---|---|
| Apathy        | + | − | − | + | − | + | − | + | − |
| Abnormal eating behaviour | + | − | + | + | + | − | + | + | − |
| Obsessive/compulsive behaviour | − | + | − | − | + | − | + | − | − |
| Loss of empathy | − | + | − | − | + | − | + | − | − |

#### Cognitive features

| Executive dysfunction | + | NK | + | + | − | NK | + | NK | + |
|------------------------|---|----|---|---|---|----|---|----|---|
| Language impairment    | + | − | + | + | + | − | + | NK | − |
| Episodic memory impairment | + | − | + | − | + | − | + | NK | − |

#### Imaging features

| Symmetry of atrophy (left = L, right = R) | R = L | NK | R = L | R = L | R > L | NK | R > L |

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### Table 2. Distribution of TDP-43 and p62 positive lesions in GRN/C9orf72 cases compared to GRN and C9orf72 cases

| Anatomical region | C9ORF72 – Case 1 | C9ORF72 – Case 2 | C9ORF72 – Case 3 | Case 2.1 | Case 2.2 | GRN – Case 1 | GRN – Case 2 | GRN – Case 3 |
|-------------------|-------------------|-------------------|-------------------|----------|----------|--------------|--------------|--------------|
| Frontal Cortex    | TDP               | p62               | TDP               | p62      | TDP      | p62          | TDP          | p62          |
| Grey Matter       | +++               | +++               | +++               | +++      | +++      | +++          | +++          | +++          |
| White Matter      | ++                | ++                | +                 | +        | +        | +            | +            | +            |
| Temporal Cortex   | TDP               | p62               | TDP               | p62      | TDP      | p62          | TDP          | p62          |
| Grey Matter       | ++                | ++                | +                 | +        | +        | +            | +            | +            |
| White Matter      | +                 | +                 | +                 | +        | +        | +            | +            | +            |
| Hippocampus       | GCL               | TDP               | p62               | TDP      | p62      | TDP          | p62          | TDP          |
| GCL       | +++               | +++               | ++                | ++       | +        | +            | +            | +            |
| CA1       | +                 | +++               | +                 | +        | +        | +            | +            | +            |
| CA2       | 0                 | +++               | 0                 | +        | 0        | +            | 0            | 0            |
| CA3       | 0                 | +                 | 0                 | +        | 0        | +            | 0            | 0            |
| CA4       | 0                 | +++               | 0                 | +        | 0        | +            | 0            | 0            |
| Subiculum    | +                 | ++                | +                 | +        | +        | +            | +            | +            |
| Entorhinal    | +                 | ++                | +                 | +        | +        | +            | +            | +            |
| Fusiform     | +                 | ++                | +                 | +        | +        | +            | +            | +            |
| Cerebellar white matter | 0             | +                 | 0                 | +        | +        | +            | +            | +            |
| Cerebellar GCL | 0                 | +                 | 0                 | +        | +        | +            | +            | +            |

Semi-quantitative analysis of TDP-43 and p62 positive lesions observed in three cases carrying the C9orf72 expansion repeat, three cases carrying a GRN mutation and the two family members carrying both the C9orf72 and GRN mutation. Score is an aggregate of all lesions present including neuronal cytoplasmic, neuronal intranuclear inclusions and neurites. Grading: 0 = absent; + = mild; ++ = moderate; +++ = frequent; ++++ = severe. GCL, granule cell layer.
dentate fascia, CA4 subregion of the hippocampus and the granule cell layer of the cerebellum. This was evident when the number of TDP-43 and p62 positive inclusions were assessed in a semi-quantitative manner (Table 2). The three GRN only cases only demonstrated TDP-43 positive inclusions in all regions and the p62 positive immunohistochemistry mirrored what was observed with the TDP-43. No additional p62-positive star-like inclusions were found in the hippocampus or cerebellum of the GRN only cases. In all the GRN cases the amount of TDP-43 pathology was increased in the frontal and temporal cortices as well as the number of NCIs observed in the GCL compared to case 2.1 and 2.2.

**Discussion**

In this study we describe a family that carry both a GRN pathogenic mutation and the newly identified C9orf72 hexanucleotide repeat expansion. Pathologically the two cases were classified as FTLD-TDP type A, with additional p62-positive ‘star-like’ inclusions throughout the different brain regions but predominantly in the hippocampal formation and cerebellum. The family described here were known to segregate for a causal GRN mutation. The possibility of concurrence was prompted by the histopathological findings, which were in keeping with those found in C9orf72 expansion repeat cases. The chance concurrence of the two mutations can be crudely estimated from the population frequencies of the mutations. Pathogenic GRN mutations have not been found in the healthy population whereas C9orf72 mutations are found in approximately 1:700 [24]. As we examined ~20 GRN distantly related cases post-mortem, the probability of a single chance concurrence was approximately 0.03 (20/700). A further possibility is that the concurrence of the mutations increases the penetrance of FTLD; however, in several studies; the penetrance of each mutation in isolation is high. Others have described concurrence of C9orf72 and TDP-43 mutations [23,24] and C9orf72 expansions with 2 novel missense mutations in GRN (Y294C) and in PSEN-2(1146V) [25]. A systematic study of all causal genes in neurodegeneration will be required to assess whether concurrent causal mutations are found more often than would be expected by chance alone. From a clinical perspective the possibility of concurrent mutations may lead to serious complications when providing genetic testing for affected families. Following the discovery of the link between GRN and FTLD, many at-risk family members have come forward for predictive genetic testing, often with influences on major life decisions. The subsequent discovery of a second causal mutation in the family is clearly a tragedy for those having completed such a process and seemingly freed from risk of the family disorder. From a genetic counselling perspective it would therefore be of importance to clarify whether these concurrences are extremely rare and purely chance events, or if they are found more frequently than would be expected because of increased penetrance.

Pathologically both cases demonstrated TDP-43 deposition with numerous NCIs, occasional intanuclear inclusions and short neurites, which are in keeping with the FTLD-TDP type A subtype, previously observed in both GRN mutation and C9orf72 expansion repeat cases. The ‘star-like’ p62 inclusions, found throughout different brain regions, which were readily visible in areas lacking TDP-43 pathology, are characteristic for cases with C9orf72 expansion repeat. Additional pathology was confirmed in one of our cases in the form of NFT pathology corresponding to Braak and Braak stage II and this case also showed evidence of cerebrovascular disease, which was thought to be ‘age-related’ as this patient was 11 years older at death than the patient without additional pathology.

It remains to be determined how the C9orf72 expansion repeat length affects the clinical phenotype and the underlying pathology once the expansion repeat can be sized accurately. The two cases analysed in this study demonstrated different repeat lengths, 4153 in case 1 and 3316 in case 2. However, it is of note that in one case brain tissue was used for DNA extraction while peripheral lymphocytes in the other, which may be relevant as it is not known whether the C9orf72 repeat length is different when it is determined in peripheral tissue or brain tissue.

Both our cases carry two confirmed pathogenic mutations, which raises the question of which of the two genetic abnormalities is driving the underlying pathology or whether both contribute to it. Clinically the main features of this pedigree are of progressive speech, gait and cognitive impairment. A diagnosis of FTD-MND was made in one of the cases, which would be most consistent with an expansion in C9orf72, although there are rare cases of FTD-MND in association with GRN mutations [25]. Brain imaging showed a symmetrical pattern of atrophy, which would be in keeping with an expansion in C9orf72, whereas GRN mutations show a more asymmetrical pattern of atrophy [25].
In both of our cases the TDP-43 pathology corresponds to FTLD-TDP type A, which is the pattern seen in cases with GRN mutations [26], although cases with C9orf72 repeat expansion alone can also be associated with this FTLD-TDP subtype [27]. The Cys31fs GRN mutation, as identified in the family reported here, is a tetranucleotide insertion in the coding region causing frameshift and premature translation termination, resulting in nonsense-mediated mRNA decay, causing a loss of GRN function rendering the protein less active or ineffective.

Neurologically normal controls have been identified to carry expansion greater than 45 repeats [25]. However, to date, no data from Southern blot analysis are available in control cases, which would provide information about the size of the expansion repeat. Furthermore, in control cases carrying a C9orf72 repeat expansion no pathological data are available either about whether TDP-43 and/or p62 pathology is present in such cases, which would indicate that they represent a ‘preclinical disease stage’. Therefore without more knowledge of the pathogenic repeat length the C9orf72 hexanucleotide repeat expansion and data of detailed TDP-43 and p62 immunohistochemical studies in ‘normal’ control cases it would be difficult to suggest the C9orf72 expansion is merely a risk factor contributing to the susceptibility to the disease in our cases as it has been suggested for another GRN mutation case combined with C9orf72 expansion [28]. However, in that case it remains to be proven if the missense GRN mutation is pathogenic or not.

In conclusion, the cases presented here carry ‘double’ pathogenic mutations, both of which have an effect on the cellular pathways finally manifesting in TDP-43 pathology. Future studies are required to understand the precise mechanisms and contribution the two different genetic abnormalities make in such cases.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. TDP-43 pathology in GRN/C9orf72 cases. Cases 2.1 and 2.2 both demonstrated FTLD-TDP type A pathology. Neuronal cytoplasmic inclusions were found in the granule cell layer of the hippocampal formation (A, arrow). Occasional neuronal intranuclear inclusions were found in the frontal cortex (B, arrow) together with neuronal cytoplasmic inclusions mainly found in layer two of the cortex (C). Many short dystrophic neurites were also found (D).

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