

# Symmetrical Corticobasal Syndrome Caused by a Novel c.314dup *Progranulin* Mutation

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**Abstract** Corticobasal syndrome (CBS) is characterised by asymmetrical parkinsonism and cognitive impairment. The underlying pathology varies between corticobasal degeneration, progressive supranuclear palsy, Alzheimer's disease, Creutzfeldt–Jakob disease and frontotemporal lobar degeneration sometimes in association with *GRN* mutations. A 61-year-old male underwent neurological examination, neuropsychological assessment, MRI, and HMPAO-

SPECT at our medical centre. After his death at the age of 63, brain autopsy, genetic screening and mRNA expression analysis were performed. The patient presented with slow progressive walking disabilities, non-fluent language problems, behavioural changes and forgetfulness. His family history was negative. He had primitive reflexes, rigidity of his arms and postural instability. Later in the disease course he developed dystonia of his left leg, pathological crying, mutism and dysphagia. Neuropsychological assessment revealed prominent ideomotor and ideational apraxia, executive dysfunction, non-fluent aphasia and memory deficits. Neuroimaging showed symmetrical predominant frontoparietal atrophy and hypoperfusion. Frontotemporal lobar degeneration (FTLD)-TDP type 3 pathology was found at autopsy. *GRN* sequencing revealed a novel frameshift mutation c.314dup, p.Cys105fs and *GRN* mRNA levels showed a 50% decrease. We found a novel *GRN* mutation in a patient with an atypical (CBS) presentation with symmetric neuroimaging findings. *GRN* mutations are an important cause of CBS associated with FTLD-TDP type 3 pathology, sometimes in sporadic cases. Screening for *GRN* mutations should also be considered in CBS patients without a positive family history.

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## Introduction

Corticobasal syndrome (CBS) is characterised by progressive asymmetric rigidity and apraxia, accompanied by additional signs of cortical and extrapyramidal dysfunction such as alien limb phenomenon, cortical sensory loss, myoclonus, bradykinesia and focal dystonia unresponsive to dopaminergic treatment (Boeve et al. 2003). Although

corticobasal degeneration (CBD) is the most frequent underlying pathology of CBS, other neuropathological causes, including frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), progressive supranuclear palsy (PSP) and Creutzfeldt–Jakob disease have been reported (Boeve et al. 1999; Wadia and Lang 2007; Tartaglia et al. 2010).

Although family history is usually negative in CBS (Spina et al. 2007), a familial form has been described which can be caused by *progranulin* (*GRN*) mutations (Wadia and Lang 2007; van Swieten and Heutink 2008). *GRN* mutations show a high phenotypic variability reflected by diagnoses of frontotemporal dementia, progressive non-fluent aphasia or AD within the same families (Gass et al. 2006; Beck et al. 2008; Le Ber et al. 2008; van Swieten and Heutink 2008; Kelley et al. 2009). The underlying pathology of *GRN* mutations is FTLD with TAR DNA-binding protein of 43 kDa (TDP-43) positive neuronal cytoplasmic and intranuclear inclusions (Gass et al. 2006; Whitwell et al. 2007; Beck et al. 2008; Kelley et al. 2009) which is known as FTLD-TDP type 3 (Cairns et al. 2007).

In this report, we describe the clinical, radiological, pathological and genetic features of a 61-year-old patient with an initial presentation of symmetric CBS caused by a novel *GRN* c.314dup mutation in the absence of a positive family history.

## Materials and Methods

### Subjects

A 61-year-old male was referred to the outpatient clinic of our neurology department. On the basis of neurological examination, neuropsychological assessment, brain MRI and single photon emission computed tomography with  $^{99m}\text{Tc}$ -hexamethyl propyleneamine oxine–single-photon emission computed tomography (SPECT), he was diagnosed with atypical parkinsonism with dementia. He died from bronchopneumonia at the age of 63.

### Neuropathological Analysis

Brain autopsy was performed by the Netherlands Brain Bank according to their legal and ethical code of conduct. Paraffin-embedded sections from all brain regions were stained with haematoxylin and eosin, Bodian, methenamine silver, and Congo Red. Immunostaining with antibodies for tau (Innogenetics), ubiquitin (DAKO),  $\beta$ -amyloid (DAKO),  $\alpha$ -synuclein (Zymed Laboratories), p62 (BD Biosciences Pharmingen), FUS (Sigma-Aldrich anti-FUS) and TDP-43 (Biotech) was performed. Neuropathological diagnosis was made by a neuropathologist (A.J.M.R.).

### Genetic Analysis

DNA was extracted from peripheral blood using the DNA Blood Kit Special (Chemagen, Baesweiler), which is based on DNA extraction and purification with magnetic beads (Smit et al. 2000). All coding exons (2–13) and exon/intron boundaries of *GRN* (NM\_002087.2) were amplified from genomic DNA by PCR and directly sequenced in both strands using the ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA). Data were analysed using SeqScape software (version 2.6; Applied Biosystems).

### mRNA Expression Analysis

Total RNA was extracted from cerebellar brain tissue using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA), and quality and quantity was assessed on the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples were normalised to 50 ng/ $\mu\text{l}$ , and using 200 ng as the template, a reverse transcription reaction was performed using a 1:1 mix of random hexamers and oligo(dT) primers and the SuperScript III system (Invitrogen, Carlsbad, CA). Gene expression assays were ordered from Applied Biosystems for *GRN* (Hs00963703\_g1) and *GAPDH* (Hs00266705\_g1) as an endogenous control. Real-time PCR was performed on an ABI 7900 using the TaqMan method. Reactions contained 1  $\mu\text{l}$  of cDNA amplified with 0.25  $\mu\text{l}$  primer/probe mix and 2.5  $\mu\text{l}$  TaqMan 2 $\times$  Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The cycling parameters recommended by the manufacturer were followed. All samples were run in triplicate and normalised to *GAPDH*. The carboxyfluorescein (FAM)-fluorescent signal was analysed using the SDS2.2.2 software, and relative quantities of *GRN* mRNA were determined using the  $\Delta\Delta\text{ct}$  method.

## Results

### Clinical, Neuropsychological and Imaging Features

A 61-year-old male barber presented with a 2-year duration of progressive walking disability, frequent falls and difficulty with rising from a chair. This motor decline was later followed by loss of initiative, emotional lability, more compliant behaviour and increasing forgetfulness. He made inappropriate sexual remarks, and his speech became non-fluent with perseverations, word-finding difficulties and comprehension deficits. Financial matters were taken over by his wife. Furthermore, he needed assistance for dressing and personal hygiene and was occasionally incontinent of urine and faeces. His symptoms were unresponsive to galantamine and levodopa. The patient's family history was

negative for neurodegenerative disorders. His father died at the age of 72 from a stroke and his mother at the age of 53 due to liver cancer.

Initial neurological examination showed normal eye movements, a mask-like facial expression, hypokinetic dysarthria, positive primitive reflexes, subtle axial rigidity, hypokinetic-rigid syndrome with cogwheeling of the upper extremities, mildly increased tone of the lower extremities, postural instability and impaired tandem gait.

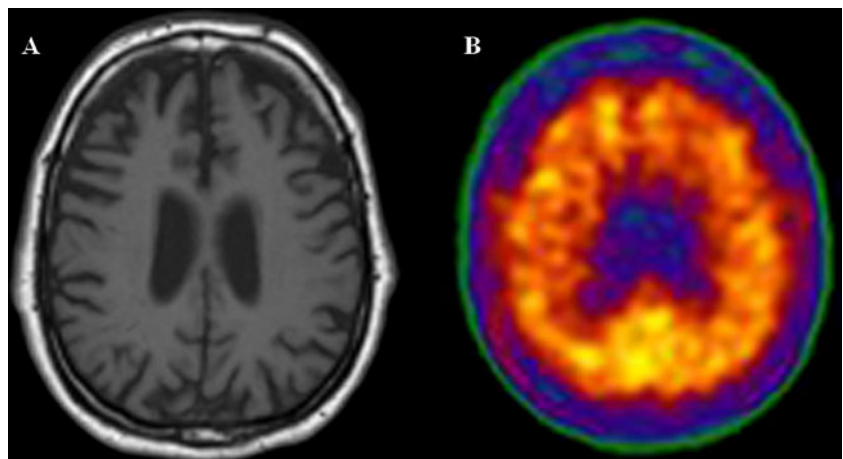
Neuropsychological testing revealed a Mini Mental State Examination score of 24/30 with intact orientation in time and place. The patient was highly distractible and exhibited sparse spontaneous speech with stuttering and perseverations. Ideomotor and ideational apraxia were prominent, the patient did not succeed in imitation, constructional tasks, and rapid alternating hand movements. Furthermore, he had severely impaired executive and memory functions, poor semantic word fluency and naming deficits but intact visuospatial functions. Neuroimaging revealed mild symmetrical cerebral atrophy, most prominent frontoparietal and perisylvian on MRI and symmetrical frontoparietal hypoperfusion on SPECT, which is shown in Fig. 1.

The patient's condition further declined over the subsequent years. Neurological examination at a follow-up visit 1 year later revealed slowed saccadic eye movements, severe symmetrical rigidity of the upper extremities, an increased tone with dystonic flexion posturing of the left leg and pathological crying. Finally, he developed mutism and dysphagia, which did not respond to amitriptyline treatment, and he died from bronchopneumonia at the age of 63 years.

#### Neuropathological Examination

Gross brain (1,327 g) examination showed mild generalised atrophy of the cortex with atrophy of caudate nucleus and putamen and normal pigmentation of the substantia nigra.

**Fig. 1** Mild symmetric frontoparietal atrophy on MRI (a) and symmetric parietal hypoperfusion on SPECT scan (b)



Microscopic examination revealed moderate to severe neuronal loss, gliosis and spongiosis in the frontal and parietal cortices but mild changes in the temporal cortex, hippocampus and basal ganglia. Immunohistochemistry showed TDP-43-positive dystrophic neurites, neurocytoplasmic and intranuclear inclusions most prominent in the second superficial layer of the frontal and parietal cortices and to a lesser extent in the temporal cortex, hippocampus and basal ganglia, consistent with a diagnosis of FTLTDP type 3 (Fig. 2) (Cairns et al. 2007).

#### Genetic Analysis

After receiving the autopsy results, we screened *GRN* and identified a novel c.314dup, p.Cys105fs mutation. This frameshift mutation causes premature termination of the coding sequence, which is likely to result in loss of function of Granulin.

#### mRNA Expression Analysis

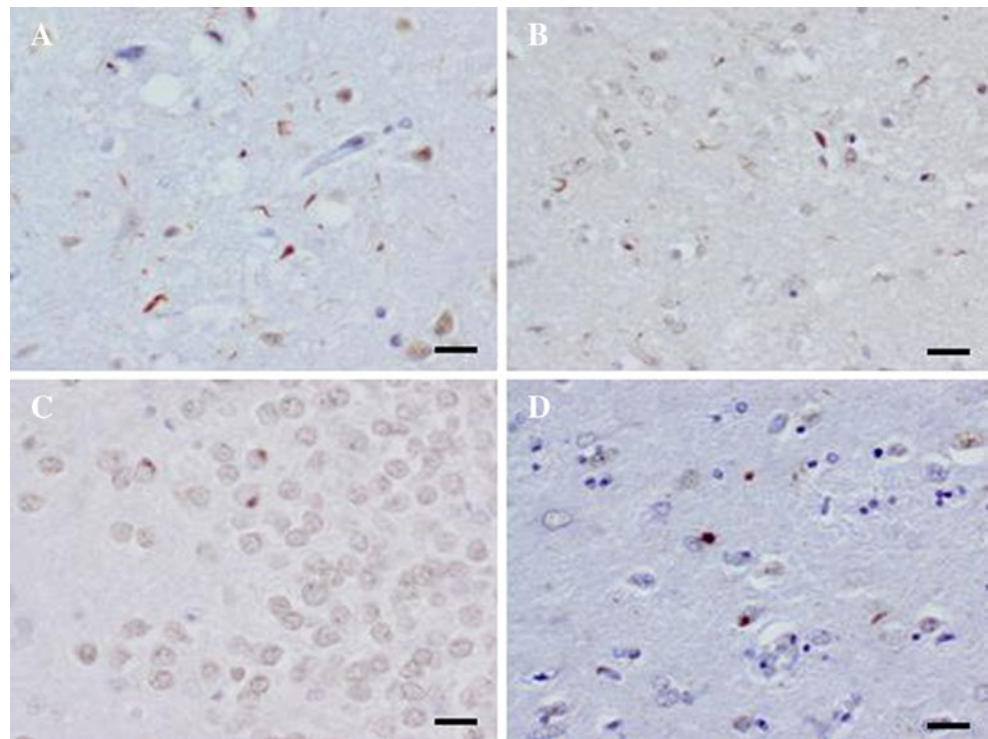
*GRN* mRNA expression analysis in cerebellar tissue from this patient showed a 50% decrease in *GRN* mRNA levels compared to a pathologically-confirmed normal control, confirming the pathogenicity of the mutation.

#### Discussion

We described a patient with an atypical CBS presentation with symmetrical neuroimaging findings with FTLTDP type 3 pathology due to a novel c.314dup, p.Cys105fs mutation in the absence of a positive family history.

The clinical presentation of our case showed strong similarities with the symmetric CBD described by Hassan et al., except for the presence of ideomotor and ideational apraxia (Hassan et al. 2010). The symmetric rigidity and the

**Fig. 2** TDP-43-positive dystrophic neurites in the frontal cortex (a) a TDP-43-positive neuronal intranuclear inclusion in the parietal cortex (b), and TDP-43-positive neuronal cytoplasmic inclusions in hippocampus (c), and caudate nucleus and putamen (d). Scale bar=20  $\mu$ m



absence of alien hand phenomenon and myoclonus did not fit with the clinical diagnosis CBS in the initial phase of the disease (Boeve et al. 2003). Moreover, the symmetric pattern of frontoparietal atrophy on MRI and parietal hypoperfusion on SPECT scan was inconsistent with the typical asymmetric distribution in CBS but was in line with the observations in symmetric CBD (Boeve et al. 2003; Whitwell et al. 2010a; Hassan et al. 2010). In a later stage of the disease, our patient developed dystonia in the left leg, which made the clinical diagnosis CBS more likely (Boeve et al. 2003). However, we did not have the opportunity to visualize an asymmetric pattern of atrophy, as an attempt to obtain additional neuroimaging was unsuccessful.

The neuropathological findings of the present case were similar to those in the case by Spina et al., which showed TDP-43 pathology most prominent in parietal and frontal cortex and to a lesser extent in the temporal cortex, with involvement of the basal ganglia (Spina et al. 2007). Their patient had a more typical CBS presentation with an alien hand phenomenon and marked asymmetry of symptoms as well as neuroimaging findings, which is more often seen in CBS patients with *GRN* mutations (Masellis et al. 2006; Spina et al. 2007; Benussi et al. 2008). The combination of parkinsonism, apraxia, behavioural changes, non-fluent speech and memory impairment in our patient could retrospectively have indicated a *GRN* mutation because these symptoms have been more frequently observed in FTLD patients with *GRN* mutations (Beck et al. 2008; Le Ber et al. 2008; Kelley et al. 2009).

The absence of a positive family history in the current case emphasises the possibility of reduced penetrance of *GRN* mutations or concealed family history due to the wide range in onset age of *GRN* mutations (Gass et al. 2006; Le Ber et al. 2007; Whitwell et al. 2007; Seelaar et al. 2008). We only screened for *GRN* mutations after the observation of FTLD-TDP type 3 pathology at autopsy. This case therefore underlines that genetic screening should be considered even when family history appears to be negative. The novel *GRN* frameshift mutation in this patient led to a premature stop codon and thereby to loss of function of Granulin, which was confirmed by reduced mRNA expression levels in the cerebellar tissue. Recent studies have shown reduced Granulin levels in the serum of patients with *GRN* mutations (Coppola et al. 2008; Ghidoni et al. 2008; Finch et al. 2009; Sleegers et al. 2009; Schofield et al. 2010). We could not confirm this in our patient, however, since his serum was not available.

Based on the clinical presentation of this patient, we did not predict FTLD-TDP pathology. Although non-fluent aphasia and parkinsonism can be seen in FTLD-TDP, an asymmetric presentation and pattern of atrophy is associated with all underlying pathologies of CBS, including CBD, PSP, FTLD and AD (Whitwell et al. 2010a). In addition, the extension of atrophy into the frontal cortex is typically asymmetric in patients with CBS with FTLD-TDP pathology, including those with *GRN* mutations (Whitwell et al. 2010a; Beck et al. 2008; Le Ber et al. 2008; van Swieten and Heutink 2008; Rohrer et al. 2010; Whitwell et al. 2010b). Therefore the

present case is unique by its atypical CBS presentation and symmetric atrophy pattern associated with FTLTD-TDP type 3 pathology with a novel *GRN* mutation.

To conclude, we found a novel *GRN* mutation in a patient with an atypical CBS presentation with symmetric neuroimaging findings without a positive family history. *GRN* mutations are an important cause of CBS associated with FTLTD-TDP pathology. Genetic screening should also be considered in CBS patients with a negative or concealed family history.

**Competing interests** None.

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