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Clinical variability and onset age modifiers in an extended Belgian *GRN* founder family

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ABSTRACT

We previously reported a granulin (*GRN*) null mutation, originating from a common founder, in multiple Belgian families with frontotemporal dementia. Here, we used data of a 10-year follow-up study to describe in detail the clinical heterogeneity observed in this extended founder pedigree. We identified 85 patients and 40 unaffected mutation carriers, belonging to 29 branches of the founder pedigree. Most patients (74.4%) were diagnosed with frontotemporal dementia, while others had a clinical diagnosis of unspecified dementia, Alzheimer's dementia or Parkinson's disease. The observed clinical heterogeneity can guide clinical diagnosis, genetic testing, and counseling of mutation carriers. Onset of initial symptomatology is highly variable, ranging from age 45 to 80 years. Analysis of known modifiers, suggested effects of *GRN* rs5848, microtubule-associated protein tau H1/H2, and chromosome 9 open reading frame 72 G_4C_2 repeat length on onset age but explained only a minor fraction of the variability. Contrary, the extended *GRN* founder family is a valuable source for identifying other onset age modifiers based on exome or genome sequences. These modifiers might be interesting targets for developing disease-modifying therapies.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a heterogeneous group of neurodegenerative disorders. Three clinical subtypes have been defined: the behavioral variant of frontotemporal dementia (bvFTD) and the nonfluent and semantic variant of primary progressive aphasia (PPA) (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). The pathological hallmark of FTLD is neuronal loss in the frontal and temporal lobes of the brain (Neary et al., 2005). FTLD is a proteinopathy and, based on the nature of the inclusion proteins, 5

See Appendix for Members of the Belgian Neurology (BELNEU) Consortium.

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pathological subtypes are recognized. FTLD-tau, with inclusions of microtubule-associated protein tau (*MAPT*), and FTLD-Tat activating regulatory (TAR)DNA-binding protein (FTLD-TDP) are the most frequent pathological diagnoses (Mackenzie and Neumann, 2016). Causal mutations have been identified in 6 genes, namely granulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*), *MAPT*, TANK-binding kinase 1 (*TBK1*), valosin-containing protein (*VCP*), and charged multivesicular body protein 2B (*CHMP2B*) (Baker et al., 2006; Cruts et al., 2006; Dejesus-Hernandez et al., 2011; Freischmidt et al., 2015; Gijselinck et al., 2012, 2015; Hutton et al., 1998; Poorkaj et al., 1998; Pottier et al., 2015; Renton et al., 2011; Skibinski et al., 2005; Spillantini et al., 1998; Watts et al., 2004). Variations in these genes have been suggested to modify disease risk, such as the common variation rs5848 in *GRN*, the H1/H2 haplotype of *MAPT*, and intermediate alleles of 7–24 repeat units of the *C9orf72* G_4C_2 repeat (e.g., [Benussi et al., 2014; Borroni et al., 2005; Gijselinck et al., 2012; Rademakers et al., 2008; Verpillat et al., 2002]). In addition, a genome-wide association study identified variations in *TMEM106B* as risk factors for FTLD-TDP (Van Deerlin et al., 2010).

GRN mutations explain 10%–25% of all and 3%–26% of familial FTD patients (http://www.molgen.vib-ua.be/FTDMutations; Cruts et al., 2012; Sieben et al., 2012). The majority are heterozygous loss-of-function mutations that lead to premature stop codons triggering degradation of the mutant transcript. Consequently, reduced GRN levels are measured in blood and cerebrospinal fluid (CSF) (Finch et al., 2009; Ghidoni et al., 2008; Sleegers et al., 2009). In brain, FTLD-TDP pathology type A is typically present (Mackenzie and Neumann, 2016). Clinical phenotypes are diverse, even between patients carrying the same mutation, and include FTD, Alzheimer's dementia (AD), Parkinson's disease (PD), and corticobasal syndrome (e.g., [Brouwers et al., 2007; Kelley et al., 2009]). Mutation carriers show a high interfamilial and intrafamilial variability in onset age ranging from 35 to 89 years (Cruts and Van Broeckhoven, 2008; Cruts et al., 2012; Van Swieten and Heutink, 2008). This suggests that modifiers influence the disease onset and clinical appearance. Variations near sortilin (*SORT1*), a receptor of GRN, and prosaposin (*PSAP*) regulate GRN levels, but their effect on clinical heterogeneity remains unclear (Carrasquillo et al., 2010; Nicholson et al., 2016).

We identified in an extended Belgian FTD family a *GRN* founder mutation in the splice donor site of intron 1 (mutation alias IVS1+5 G>C, protein p.0). The mutation prevents splicing of intron 1, leading to nuclear retention and degradation of the mutant transcript, which results in haploinsufficiency (Cruts et al., 2006). Since its identification, the pedigree has been extended and additional genealogical and clinical data have been gathered. The Belgian *GRN* founder mutation is one of the most common *GRN* mutations worldwide, in addition to the p.Arg493X mutation (Rademakers et al., 2007) and the p.Thr272SerfsX10 mutation, which is the most frequent *GRN* mutation in Italy (Benussi et al., 2009, 2010; Borroni et al., 2011b). For all 3 mutations, founder effects have been described (Benussi et al., 2013; Borroni et al., 2011a; Cruts et al., 2006; Rademakers et al., 2007; van der Zee et al., 2006). The 10-year follow-up study of the Belgian *GRN* founder pedigree allowed us to describe in detail the clinical heterogeneity of the *GRN* patient carriers. The observed heterogeneity in onset age and the size of the extended pedigree provided an opportunity to investigate the potential onset age-modifying effects of known genetic modifiers of FTD risk or disease presentation.

2. Materials and methods

2.1. Study population

Index patients were ascertained in Belgium through an ongoing multicenter collaboration of neurology departments and memory clinics partnering in the BELNEU consortium. Additional patients, referred to the diagnostic service facility for genetic testing, were included. Information on family history of neurodegenerative diseases was gathered for the index patients, and relatives were asked to participate in genetic studies. Written informed consent for participation in clinical and genetic studies, and for the brain autopsy when appropriate, was obtained from participants and/or their legal guardians. The clinical study protocol and the informed consent forms for patient ascertainment were approved by the local ethics committees of each of the collaborating neurological centers. The genetic and pathological study protocols and informed consent forms were approved by the ethics committee of the University Hospital of Antwerp and the University of Antwerp, Belgium.

FTD, AD, or PD diagnoses were made in accordance with the international consensus criteria (Gelb et al., 1999; Gorno-Tempini et al., 2011; McKhann et al., 1984, 2011; Postuma et al., 2015; Rascovsky et al., 2011). Patients with a combination of behavioral and language features of FTD at presentation, without clear predominance, were denoted mixed FTD. In addition to index patients, symptomatic relatives with a clinical diagnosis carrying the founder mutation were included in the clinical study. All available clinical records were reviewed by a medical doctor of the research team (Sara Van Mossevelde). A positive familial history was defined as the presence of at least 1 first degree or 2 second degree relatives with neurodegenerative disease. The onset age was defined as the age at which first symptoms were noticed by the patient or his partner/relatives. Supplementary Fig. S1 summarizes the available biomaterials and clinical information.

2.2. Genetic screening

2.2.1. Targeted resequencing of genes involved in neurodegeneration

GRN founder mutation carriers and their family members were screened for mutations using parallel screening of a multigene panel of neurodegenerative brain disease-related genes (Agilent, https://www.agilent.com). Multiplex polymerase chain reactions (PCR) were performed for target enrichment of the coding regions of 17 causal genes and the apolipoprotein E gene (*APOE*). This was followed by equimolar pooling of the amplicon libraries and purification using Agencourt AMPureXP beads (Beckman Coulter). A universal PCR was performed to incorporate patient-specific barcodes. Samples were pooled, and massive parallel sequencing was performed in-house on an Illumina MiSeq platform. Paired-end reads were generated, and adapters were trimmed using Fastq-mcf. Reads were aligned to the reference genome hg19 with the Burrows-Wheeler Aligner MEMv0.7.5a (Li and Durbin, 2009). Variant calling and annotation was performed using GATKv2.4 UnifiedGenotyper and the GenomeComb package (McKenna et al., 2010; Reumers et al., 2012). We focused on known pathogenic mutations present within the genes amyloid beta precursor protein, presenilin 1, presenilin 2, *GRN*, *MAPT*, *TBK1*, *CHMP2B*, *VCP*, superoxide dismutase 1, TAR DNA-binding protein *(TARDBP)*, FUS RNA binding protein *FUS*, prion protein, synuclein alpha, leucine rich repeat kinase 2, parkin RBR E3 ubiquitin protein ligase (*PARKN)*, parkinsonism associated deglycase (*PARK7*), and PTEN induced putative kinase 1. We have identified 2 carriers of the p.G287S mutation in the *TARDBP* gene in branch DR404 of the founder pedigree (Table 1, Supplementary Fig. S2). Individual III.3 carried both mutations and was unaffected at 65 years. Sibling III.5 carried only the *TARDBP* mutation and was unaffected at 57 years. According to relatives, the parent II.3 showed a reduction in speech at 80 years, mutism at age 84 years, and loss of interest in the surroundings at 85 years. The spouse of II.3 died unaffected at