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The missense p.Trp7Arg mutation in *GRN* gene leads to progranulin haploinsufficiency

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Abstract. *GRN null* mutations are among the main genetic causes of frontotemporal dementia through progranulin haploinsufficiency. Most missense mutations are considered not pathogenic. The p.Trp7Arg substitution is localized within the signal peptide domain and no formal evidence for its pathogenicity has yet been provided. We identified the p.Trp7Arg substitution in three carriers with low plasma progranulin levels. This evidences that this missense mutation leads to functional haploinsufficiency and should thus be considered pathogenic. Assessing the pathogenicity of variants of unknown significance has significant implications for clinical practice, genetic counseling and future therapeutic interventions.

1. Introduction

Frontotemporal dementias (FTD) are characterized by progressive behavioral and language disorders (Rascovsky et al., 2011). An autosomal dominant pattern of transmission is present in up to 40% of cases. Heterozygous mutations in progranulin (*GRN*) gene are among the main genetic causes of FTD. Progranulin is a secreted protein involved in inflammation, tumorigenesis, wound repair and development (Ahmed et al., 2007). More than 70 pathogenic mutations have been reported so far, accounting for approximately 20% of familial FTD cases (Cruts et al., 2012) (<http://www.molgen.ua.ac.be/aDmutations/>). The vast majority of those are *null* mutations consisting of nonsense, splice-site and small insertions or deletions causing frameshift. Their consequence is the production of premature termination codons leading to the degradation of mutant mRNA by nonsense-mediated decay (Baker et al., 2006; Cruts et

al., 2006). Rare partial or complete gene deletions have also been reported (van der Zee et al., 2007; Rovelet-Lecrux et al., 2008; Clot et al., 2014). All these mutations lead to haploinsufficiency and reduced progranulin synthesis, reflected in the decrease of plasma progranulin levels (Finch et al., 2009).

Moreover, about 100 *GRN* missense variants have been identified to date. Their pathogenicity is much less evident and most are considered not pathogenic (Cruts et al., 2012). We previously reported the *GRN* p.Trp7Arg missense mutation within the signal peptide domain of the protein in a FTD patient (Le Ber et al., 2008) but no formal evidence for a clear pathogenic role was provided. We identified the p.Trp7Arg missense variant in a second unrelated family, and bring evidence for its deleterious effect in the present work.

2. Methods

2.1. Proband and family. The proband II.1 (Figure 1A) is a Caucasian patient who manifested insidious behavioral changes and engaged in stereotyped activities at age 53. He began to apply himself to unusually intense physical training and displayed compulsive shopping and snacking habits. Soon afterwards, he developed apathy, loss of empathy, indifference, social withdrawal and changes in diet with significant weight gain, while also exhibiting disinhibition with excessive familiarity. Neuropsychological testing at age 58 revealed dysexecutive syndrome with significantly impaired attention and verbal perseverations. Furthermore, the patient showed impairments in episodic memory and language with semantic deficit. Mini Mental Status Examination score was of 6/30 and Mattis Dementia Rating Scale score was of 57/144. Stereotyped answers and attentional deficit rendered further assessment impossible. Brain MRI evinced frontal, lateral temporal and parietal atrophy (Figure 1B). Moreover, ¹⁸FDG-PET revealed severe hypometabolism in dorsolateral prefrontal, orbitofrontal, cingulate cortex, temporal pole and temporo-parietal junction (Figure 1C). Neuroimaging changes were prevalent on the left. A probable behavioral variant of FTD

(bvFTD) was diagnosed. One of the patient's parents (I.1) exhibited cognitive and behavioral disorders suggestive of bvFTD at age 63, and died at age 70. Two siblings (II.2 and II.3) were asymptomatic at age 50 and age 54.

2.2. Progranulin plasma assay

Plasma progranulin level was measured with the ELISA method using the progranulin-human-Elisa kit (Adipogen, Coger SAS, France), in compliance with the manufacturer instructions. The range of normal values is above 110 µg/L in healthy individuals (Ghidoni et al., 2008; Finch et al., 2009).

2.3. Progranulin mutation analysis.

DNA was extracted from peripheral blood samples from the proband (II.1) and two relatives (II.2 and II.3) after consent for clinical and genetic research analyses was obtained. Amplified fragments of the 13 exons and exon-intron junctions of the *GRN* gene were sequenced using the Sanger method on an ABI 3730 automated sequencer using the Big Dye 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) as previously described (Le Ber et al., 2008). Additionally, Multiplex Ligation-dependent Probe amplification (MLPA) (SALSA MLPA-P275-MAPT-GRN probemix, MRC-Holland, Netherlands) was performed in the proband to exclude a whole gene deletion. This study received the approval from the local ethical committee (AP-HP Paris).

3. Results

Sequencing of *GRN* gene revealed the c.19T>C, p.Trp7Arg in the exon 2 at heterozygous state (NM_002087.2) in the proband (II.1). Two asymptomatic first-degree relatives (II.2 and II.3), investigated in a research setting, carried the same heterozygous *GRN* mutation. DNA of the affected parent and of the unaffected sibling II.4 was not available. This mutation was previously identified in an unrelated French FTD family (Le Ber et al., 2008) and is absent from public databases of controls (GnomAD, dbSNP, EVS, ESP, ExAC). The p.Trp7Arg

substitution is located in the signal peptide sequence of the protein and is thus expected to affect the translocation of the protein in the lumen of endoplasmic reticulum (ER). A whole gene deletion was excluded by MLPA in the proband.

The plasma progranulin level was low in all the three mutation carriers compared to reference levels in the normal population (Ghidoni et al., 2008; Finch et al., 2009). In the proband, the plasma level was 43 and 41 µg/L in two independent dosages at two time points. Low levels of 43 and 39 µg/L were also detected in II.2 and II.3 respectively (Figure 1A).

4. Discussion

We herein report a French family displaying FTD associated with the *GRN* missense p.Trp7Arg mutation and decreased plasma progranulin levels. The pathogenicity of most *GRN* missense variants is deemed uncertain (Cruts et al., 2012). Rare missense mutations (p.Val5Leu, p.Trp7Arg and p.Ala9Asp) in the N-terminal signal peptide domain are of particular interest. They could indeed disrupt the translocation into the ER, resulting in inefficient trafficking through the secretory pathway, mislocalization of the mature progranulin and, consequently, functional haploinsufficiency.

Functional studies have demonstrated the pathogenic nature of the p.Ala9Asp mutation identified in more than 10 unrelated FTD families (<http://www.molgen.ua.ac.be/aDmutations/>) (Cruts et al., 2012; Pinarbasi et al., 2018). The pathogenicity of p.Val5Leu and p.Trp7Arg variants remains elusive (Cruts et al., 2012). Notably, the p.Trp7Arg mutation was identified in a French patient affected by bvFTD with a family history of dementia. However, no formal evidence for a clear pathogenic role could be provided in this case (Le Ber et al., 2008) and no other families carrying this variant have been identified so far to confirm its implication in FTD. So far, the p.Trp7Arg has therefore been considered as a variant of unknown significance (VUS) and its pathogenic nature has been unclear in genetic FTD databases (Cruts et al., 2012).

In this study, we put forward compelling arguments in favor of the pathogenicity of the p.Trp7Arg mutation. First, the discovery of the mutation in a second family, unrelated to the previous one, and its absence from public databases of controls support an association with the disease. More importantly, low progranulin levels in three mutation carriers of this family demonstrate that the p.Trp7Arg substitution promotes progranulin haploinsufficiency. The two asymptomatic mutation carriers have been likely investigated during their presymptomatic phase, considering their respective ages lower than 55. Similar to the p.Ala9Asp mutation, the p.Trp7Arg mutation introduces a polar amino acid within the hydrophobic region of the signal peptide domain. This could potentially impair the interaction with the signal recognition particle, which leads nascent progranulin into the ER lumen. The progranulin plasma levels in the mutation carriers support the results of *in vitro* studies showing that the p.Trp7Arg variant reduces progranulin localization within the ER (Pinarbasi et al., 2018).

In conclusion, our study provides compelling evidence that the p.Trp7Arg missense variant in *GRN* gene has a causative role in FTD. Assessing the pathogenicity of FTD-related VUS has major implications for clinical diagnosis and genetic counseling, and is a fundamental prerequisite to directing patients and presymptomatic carriers to future therapeutic interventions.

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Figure 1. A: family pedigree. Subjects' gender has been masked for the sake of confidentiality. Black squares represent affected subjects and grey squares presymptomatic carriers. The arrow indicates the proband. AO: age at onset. AD: age at death. B: Axial T1-weighted MRI showing severe and asymmetrical frontal, temporal and parietal atrophy, predominant on the left. C: ¹⁸FDG-PET showing severe prefrontal, cingulate, temporal and parietal hypometabolism (left>right).

