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Progranulin plasma levels predict the presence of *GRN* mutations in asymptomatic subjects and do not correlate with brain atrophy: results from the GENFI study

Daniela Galimberti [a,*], Giorgio G. Fumagalli [a,b,1], Chiara Fenoglio [a,1], Sara M.G. Cioffi [a], Andrea Arighi [a], Maria Serpente [a], Barbara Borroni [c], Alessandro Padovani [c], Fabrizio Tagliavini [d], Mario Masellis [e], Maria Carmela Tartaglia [e], John van Swieten [f], Lieke Meeter [f], Caroline Graff [g,h], Alexandre de Mendonça [i], Martina Bocchetta [j], Jonathan D. Rohrer [j], Elio Scarpini [a], on behalf of the Genetic FTD Initiative (GENFI)[2]

[a] *Department of Pathophysiology and Transplantation, University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy*
[b] *Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy*
[c] *Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy*
[d] *Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Istituto Neurologico Carlo Besta, Milano, Italy*
[e] *LC Campbell Cognitive Neurology Research Unit, Department of Medicine, Division of Neurology, Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Ontario, Canada*
[f] *Department of Neurology, Erasmus Medical Center, Rotterdam, the Netherlands*
[g] *Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Division of Neurogeriatrics, Karolinska Institutet, Huddinge, Sweden*
[h] *Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden*
[i] *Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[j] *Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK*

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ABSTRACT

We investigated whether progranulin plasma levels are predictors of the presence of progranulin gene (*GRN*) null mutations or of the development of symptoms in asymptomatic at risk members participating in the Genetic Frontotemporal Dementia Initiative, including 19 patients, 64 asymptomatic carriers, and 77 noncarriers. In addition, we evaluated a possible role of *TMEM106B* rs1990622 as a genetic modifier and correlated progranulin plasma levels and gray-matter atrophy. Plasma progranulin mean ± SD plasma levels in patients and asymptomatic carriers were significantly decreased compared with non-carriers (30.5 ± 13.0 and 27.7 ± 7.5 versus 99.6 ± 24.8 ng/mL, $p < 0.00001$). Considering the threshold of >61.55 ng/mL, the test had a sensitivity of 98.8% and a specificity of 97.5% in predicting the presence of a mutation, independent of symptoms. No correlations were found between progranulin plasma levels and age, years from average age at onset in each family, or *TMEM106B* rs1990622 genotype ($p > 0.05$). Plasma progranulin levels did not correlate with brain atrophy. Plasma progranulin levels predict the presence of *GRN* null mutations independent of proximity to symptoms and brain atrophy.

Mutations in progranulin gene (*GRN*) are associated with familial forms of frontotemporal dementia (FTD). The majority of mutations cause haploinsufficiency and are associated with an extremely heterogeneous clinical presentation (Woollacott and Rohrer, 2016) and TAR DNA–binding protein-43 pathology (Neumann et al., 2006). Progranulin displays anti-inflammatory properties but can also undergo cleavage to produce granulins, which, conversely, have proinflammatory properties (Tang et al., 2011). Abnormalities of several cytokines and chemokines has been observed in cerebrospinal fluid (CSF) of *GRN* carriers compared with controls (Galimberti et al., 2015), suggesting an imbalance of specific inflammatory factors possibly related to *GRN* haploinsufficiency.

* Corresponding author at: University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, via F. Sforza, 35, 20122 Milan, Italy. Tel.: +390255033847; fax: +390255036580.
E-mail address: daniela.galimberti@unimi.it (D. Galimberti).
[1] These authors contributed equally to this work.
[2] List of GENFI consortium members in Appendix.

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Previous studies have demonstrated that patients with null mutations in *GRN* display very low plasma progranulin levels compared with sporadic FTD (Finch et al., 2009; Ghidoni et al., 2008; Sleegers et al., 2009), and that this analysis is useful to identify carriers of mutation causing haploinsufficiency, independent of the clinical presentation (Carecchio et al., 2009). A multicenter Italian study, carried out among subjects attending to a memory clinic, suggested a cutoff level of 61.55 ng/mL, which was able to identify null mutation carriers with a sensitivity of 95.8% and a specificity of 99.6% (Ghidoni et al., 2012). Despite its promising role as a biomarker for predicting the presence of a mutation and thus avoiding sequencing, which is expensive and time consuming, caution should be taken in using plasma progranulin levels to predict changes in the brain. In fact, progranulin levels are differently regulated in plasma and CSF, therefore peripheral levels may not adequately represent progranulin levels in the central nervous system (Nicholson et al., 2014; Wilke et al., 2016).

Recently, it has been demonstrated in the Genetic Frontotemporal Dementia Initiative (GENFI) study that gray matter and cognitive changes can be identified 5–10 years before the expected onset of symptoms in adults at risk of genetic FTD, including a cohort carrying *GRN* mutations (Rohrer et al., 2015). Brain atrophy in presymptomatic carriers of common FTD mutations, including *GRN*, is affected by both genetic and environmental factors such as *TMEM106B* rs1990622 polymorphism (Finch et al., 2011). This gene has been actually demonstrated to act as a genetic modifier of *GRN* mutations, influencing the age at disease onset and progranulin levels in mutation carriers (Cruchaga et al., 2011; Finch et al., 2011). Carriers of 2 copies of the minor allele of rs1990622 have a significantly reduced penetrance or the onset significantly delayed (Finch et al., 2011).

Given these premises, the main aim of this study was to test the sensitivity and specificity of progranulin plasma levels as predictors of the presence of *GRN* null mutations in asymptomatic at risk members of families with known mutations enrolled in GENFI. Additional objectives include the analysis of (1) the *TMEM106B* rs1990622 genotype as a genetic modifier, and (2) the correlation between progranulin plasma levels and cortical gray-matter atrophy in both symptomatic and asymptomatic subjects.

1. Methods

1.1. Study participants

Data for this study were drawn from the GENFI multicentre cohort study (Rohrer et al., 2015). Eight centers participated in this study from the UK, Italy, the Netherlands, Sweden, Portugal, and Canada. Inclusion and exclusion criteria have been previously described (Rohrer et al., 2015). Local ethics committees approved the study at each site and all participants provided written informed consent. For the aim of the present work, we considered participants from *GRN* families including those who had already developed symptoms as well as their at-risk relatives (which includes both mutation carriers and noncarriers). Samples from 160 Caucasian subjects belonging to 42 families were available, including 19 patients [11 females and 8 males, mean age (years) at time of inclusion ± standard deviation (SD) 64.3 ± 5.71, range (years): 55–77]; 64 asymptomatic carriers (43 females and 21 males, mean age ± SD 49.1 ± 11.1, range 26–70), and 77 noncarriers (49 females and 28 males, mean age ± SD 49.6 ± 15.3, range 19–86). In accordance with the Genfi protocol, samples were collected in the morning after an overnight fasting and processed without delay locally, likely avoiding stability issues and intra-individual variations. All subjects underwent a careful recording of demographic data, including years of formal schooling, past medical history, a standardized clinical and neuropsychological assessment, as previously published, and T1-weighted MRI scan for volumetric analysis (Rohrer et al., 2015). Characteristics of the cohort studied are summarized in Table 1.

1.2. DNA isolation

Total genomic DNA was isolated from whole blood using a Flexigene Kit (Qiagen, Hildren, Germany), according to the manufacturer instructions. The amount of DNA for each sample was determined by measuring the optical density at 260-nm wavelength using a spectrophotometer (Eppendorf AG, Wesseling-Berzdorf, Germany). DNA samples were aliquoted and stored at −20 °C until use.

1.3. Genotyping

The entire open reading frame including the noncoding exon 0 and exon-intron boundaries of exons 1–12 of the *GRN* gene was sequenced using specific primers (available upon request) on an AB3130 automated sequencer (Applied Biosystems). Chromatogram analysis was carried out using SeqScape software version 2.5 (Applied Biosystem, Foster City, CA, USA). TMEM106B rs1990622 (C/T) single nucleotide polymorphism genotyping was performed according to standard procedures (Premi et al., 2014).

1.4. Plasma sample collection and progranulin level evaluation

EDTA blood samples were allowed to sit at room temperature for a minimum of 30 minutes and a maximum of 2 hours, after collection. Separation of the clot was done by centrifugation at 1000–1300 × *g* at room temperature for 15–20 minutes. Plasma was removed and dispensed in aliquots of 400 μL into cryo-tubes. Specimens were stored at −80 °C until use.

Progranulin levels were tested with a specific ELISA kit (Adipogene, Korea), using polyclonal specific antibodies. According to the manufacturer's protocol, the test has a 5.1% CV within an assay (intra-assay precision) and 6.4% CV precision between assays (interassay precision). The limit of detection is 32 pg/mL, with an assay range of 0.063–4 ng/mL. The linearity ranges from 93% to 102%.

1.5. Statistical analysis

Descriptive statistics, including means and standard deviations, or counts and percentages were calculated. For plasma progranulin

Table 1
Characteristics of the GENFI cohort of families with *GRN* mutations

Population	Symptomatics	Asymptomatics at risk	Noncarriers
N	19	64	77
Age ± SD (range)	64.3 ± 5.71 (55–77)	49.1 ± 11.1 (26–70)	49.6 ± 15.3 (19–86)
Sex (M:F)	8:11	21:43	28:49
Mean progranulin plasma levels ± SD (ng/mL)	30.5 ± 13.0	27.7 ± 7.5	99.6 ± 24.8

Key: GENFI, Genetic Frontotemporal Dementia Initiative; SD, standard deviation.