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Plasma progranulin levels for frontotemporal dementia in clinical practice: a 10-year French experience

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ABSTRACT

GRN mutations are frequent causes of familial FTD. Although there is no clear consensual threshold, plasma progranulin levels represent an efficient biomarker for predicting *GRN* mutations when decreased. We evaluated plasma levels to determine whether it could also predict age at onset (AAO), clinical phenotype or disease progression in 160 *GRN* carriers. Importantly, progranulin levels were influenced by gender, with lower levels in male than in female patients in our study. Although we found no correlation with AAO or with clinical phenotype, we confirmed that decreased level predicts *GRN* mutations, even in presymptomatic carriers more than four decades before disease onset. We also provided first evidence for the stability of levels throughout longitudinal trajectory in carriers, over a 4-year time span. Finally, we confirmed that progranulin levels constitute a reliable, cost-effective marker, suitable as a screening tool in patients with familial FTD, and more broadly in patients without family history or with atypical presentations who are less likely to be referred for molecular diagnosis.

KEY WORDS

frontotemporal dementia; progranulin (*GRN*); plasma progranulin levels; *C9orf72*; frontotemporal lobar degeneration.

1. INTRODUCTION

Frontotemporal degeneration (FTD) is an umbrella term for a spectrum of clinically, genetically and pathologically heterogeneous neurodegenerative disorders. Major FTD clinical phenotypes include: behavioral variant frontotemporal dementia (bvFTD)(Rascovsky et al., 2011), nonfluent and semantic variants of primary progressive aphasia (PPA)(Gorno-Tempini et al., 2011), progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS)(Mathew et al., 2012). Frontotemporal lobar degeneration with TAR DNA-binding protein TDP-43 inclusions (FTLD-TDP) is the major pathological subtype accounting for 50% of cases(Mackenzie and Neumann, 2016). Familial forms of FTLD-TDP are mostly related to *GRN* gene mutations and *C9orf72* repeat expansion(Mackenzie and Neumann, 2016).

GRN loss-of-function mutations are among the most frequent genetic causes, responsible for 20% of familial FTD. So far, more than 100 deleterious *GRN* mutations have been identified (<http://www.molgen.ua.ac.be>)(Cruts et al., 2012; Moore et al., 2020), most leading to progranulin haploinsufficiency(Baker et al., 2006; Cruts et al., 2006; Moore et al., 2020). Progranulin is a ubiquitous protein involved in several biological pathways including development, neuroinflammation, tumorigenesis, degradation-lysosomal pathway and neuronal survival(Cenik et al., 2012). It is secreted in biological fluids and can be measured in patient's plasma and cerebrospinal fluid (CSF). Plasma progranulin level was identified as an efficient marker for predicting *GRN* gene mutations(Ghidoni et al., 2008; Meeter et al., 2016) and deletions when decreased or undetectable(Calvi et al., 2015; Clot et al., 2014; Galimberti et al., 2018b). Previous studies also demonstrated the reliability of reduced plasma progranulin level in asymptomatic relatives carrying *GRN* mutations(Finch et al., 2009; Galimberti et al., 2018b), which strengthened the interest for this biomarker as a tool for monitoring therapeutic trials based on enhancing progranulin levels approaches(Galimberti et

al., 2018a). However, how plasma progranulin levels evolve during disease progression in *GRN* carriers is still unknown and studies assessing the longitudinal trajectory of progranulin in plasma over time are lacking.

GRN mutations are associated with a wide range of age at onset (AAO), from the 40s to over 80s, and produce a variable clinical spectrum dominated by bvFTD before PPA and CBS (Le Ber et al., 2008)(Moore et al., 2020). Accumulating evidence suggested that genetic modifiers such as transmembrane protein 106 B (*TMEM106B*) single nucleotide polymorphisms could modulate progranulin expression, and, notably, the minor G-allele of rs1990622 was established as protective in homozygous state (Cruchaga et al., 2011; Finch et al., 2011; Van Deerlin et al., 2010). However, mechanisms underlying the phenotypic variability in *GRN* carriers are still largely unknown and biological factors predicting disease heterogeneity remain to be identified.

Predicting AAO and clinical phenotypes of *GRN* carriers is a major issue for genetic counseling, to deliver accurate information to presymptomatic carriers, and in perspective of upcoming therapeutic trials. In this study, we aimed to assess whether progranulin expression in plasma could be used as a biological marker to predict the AAO, clinical phenotype, disease progression and evaluated the longitudinal trajectory of plasma progranulin levels over time in a large population of 160 *GRN* mutation carriers. We also evaluated how plasma progranulin physiologically evolve with aging in controls, and determined analytical performances of the test, in order to optimize and generalize its use in clinical practice.

2. MATERIAL AND METHODS

2.1 Subjects' selection

Our study population consisted of FTD patients with *GRN* gene mutations, presymptomatic *GRN* mutation carriers, and controls negative for *GRN* mutations. FTD patients have been

recruited in a tertiary memory clinic (Institut de la Mémoire et de la Maladie d'Alzheimer, IM2A, Pitié Salpêtrière hospital, Paris, France) and through a national network of expert centers on FTLN/FTLN-ALS (Inserm RBM 02-059). Their biological samples and clinical data have been collected since 1998, as described previously (Le Ber et al., 2006). Between 1998 and 2019, 191 patients carrying *GRN* mutations were identified by these centers. Their genetic status was established as previously described (Le Ber et al., 2008). All participants signed an informed consent for clinical genetic study approved by local ethical committee.

Plasma progranulin dosage is routinely used in clinical practice in France, since 2009 by all the French network's centers. Amongst the 191 *GRN* carriers, 129 were recruited during the ten-year period between 2009 and 2019, and therefore had undergone plasma progranulin measurement. Those 129 patients (109 probands, 20 relatives) were included in this study. They consisted in 85 patients with bvFTD, 31 PPA, 11 CBS and 2 patients with rare clinical presentations mimicking dementia with Lewy bodies (DLB) and posterior cortical atrophy (PCA). Additionally, plasma progranulin has been measured in 31 presymptomatic *GRN* mutation carriers described elsewhere (Caroppo et al., 2015), which were included in the current study. All *GRN* carriers were European (mostly French) except seven African patients. Most had frameshift (n=74), nonsense (n=55), splice-site mutations (n=24) or exonic deletions (n=3) (see supplementary table 1). Two carried the c.1A>G, p.M1? mutation and two had the c.19T>C, p.Trp7Arg mutation in the signal peptide (Saracino et al., 2019). The list of mutations is provided in Supplementary Table 1. Our control group (n=133) consisted of healthy controls (HC, n=71) and FTD patients carrying *C9orf72* repeat expansion (FTD-*C9orf72*, n=62), all negative for *GRN* mutations.

Longitudinal plasma samples were available in a subset of 112 individuals (73/129 *GRN* carriers and 39/133 controls) with a mean follow-up period between the first and the second sample of 12.7±11.8 months; fewer individuals had more than two samples over time (see

Supplementary Table 2). Among them, 32 (15 *GRN* carriers and 17 controls) had a remarkably long follow-up period, close to four years between the first and the last plasma progranulin measure.

2.2 Laboratory methods

2.2.1 Plasma progranulin dosage, precision and coefficient of variation of the ELISA method

Overall, we analyzed progranulin levels in a total of 458 plasma samples, consisting of 270 baseline and follow-up samples of patients and presymptomatic *GRN* carriers, as well as of 188 samples of *GRN*-negative controls (Supplementary Table 2). Blood samples have been collected on EDTA using the same protocol for all participants. All samples have been centralized in the Pitié-Salpêtrière laboratory. EDTA samples were centrifuged at 3500 rpm for 20 minutes at + 4°C, were aliquoted by fraction of 500 µL and then frozen in polypropylene tubes at -80°C until assay. Plasma measurements were performed using standardized procedure, blinded to mutation status. Progranulin levels were measured using the enzyme-linked immunosorbent assay (ELISA) with the progranulin-human-Elisa kit (Adipogen, *Coger SAS*, France), according to manufacturer's instructions.

Preliminarily to any analysis, we evaluated the coefficient of variation (CV%) within an assay (intra-assay variability or within-run precision) by analyzing the same control sample on 10 plates in the same run (mean concentration 146.2 ng/mL), and between assays precision (inter-assay variability) by analyzing the same control sample (mean concentration 167.4 ng/mL) on ten different experiments on independent batches. Median intra-assay coefficient of variation was 7.6% and inter-assay variability was 9.5%. A cut-off of 15% was considered acceptable for CV%. All the plasma samples were analyzed in duplicates, and progranulin measurement was repeated when CV% between duplicates was higher than 15%.

2.2.2 *TMEM106B* rs1990622 genotyping

We studied whether A or G alleles of rs1990622, a single nucleotide polymorphism (SNP) of *TMEM106B* gene, influence plasma progranulin levels or age at onset. The SNP rs1990622 was genotyped on DNA extracted from blood lymphocytes of 66 European *GRN* carriers (41 patients, 25 presymptomatic carriers) included in this study using the Sanger method, as previously described (Lattante et al., 2014).

2.3 Statistical analysis

We used the coefficient of variation (CV%) to evaluate intra-individual variability of plasma progranulin levels, defined as two different samples from the same individual obtained over a time span, in 39 *GRN*-negative controls. Receiver-Operating characteristic (ROC) curve and Youden index were used to determine the optimal cut-off value, sensitivity (Se) and specificity (Sp) of progranulin in plasma in the studied population.

We compared plasma progranulin levels in participants according to gender, AAO, phenotype (bvFTD or PPA or CBS), *GRN* mutation characteristics (nonsense, frameshift or splice site mutation; exonic localization), and *TMEM106B* SNP. The continuous variables were tested for normality of distribution with Shapiro-Wilk test. Demographic characteristics, plasma progranulin levels and genetics (presence of *GRN* mutations, *TMEM106B* alleles and genotype frequencies) were compared between participants (*GRN* patients, *GRN* presymptomatic carriers and controls) using Chi-squared tests, Student's T test, Wilcoxon-Mann-Whitney test, Kruskal-Wallis test, ANOVA, followed by Bonferroni post-hoc analyses for multiple comparisons. Correlations between plasma progranulin levels and age were calculated with the Spearman's rank coefficient (rS). Linear regression was used to assess the association between *TMEM106B*, AAO and plasma progranulin levels.

The longitudinal progression rate of plasma progranulin levels was measured using relative annualized changes, to account for different follow-up length since not all participants were assessed at the same time point. Relative annualized progression rates of plasma progranulin levels were calculated as the total change in plasma progranulin standardized by baseline progranulin and divided by the follow-up duration between the two time points. The results are expressed as percentage per year. We separately analyzed data from a subgroup of 32 individuals with the longest follow-up period (>4 years between measures). We used a fitted regression line to assess the relationship between plasma progranulin levels and time from disease onset in *GRN* mutation carriers. Time from disease onset was calculated using the real AAO for affected *GRN* carriers, whereas the mean familial AAO was used to estimate time to expected onset in presymptomatic *GRN* carriers as previously reported (Rohrer et al., 2015). Data were analyzed with the statistical software SPSS 25.0 (SPSS Inc., Chicago, IL). A *p*-value of 0.05 was set for statistical significance.

3. RESULTS

Demographic data and plasma progranulin levels of all participants are shown in Table 1 and Figure 1.

3.1 Plasma progranulin levels in controls: variation with age and over time

We have measured plasma progranulin levels in 133 controls consisting of 71 HC and 62 FTD-*C9orf72* (Table 1 and Figure 1). Median plasma progranulin levels were 122 [IQR: 101-158] ng/mL in the entire group, and were similar in HC (126.0 [98-158]) and FTD-*C9orf72* (120.5 [101.7-158]), *p*=0.86. There was no correlation between progranulin levels and age at sampling in the entire group ($r_s=0.028$, *p*=0.76), and especially in the 71 HC ($r_s=0.016$, *p*=0.90) (see Supplementary Figure 1). Levels were lower in males (117.5 ng/mL, [IQR:

102.0-154.5]) compared to females (128.0 ng/mL, [IQR: 96.8-162.3]) in HC, without reaching the statistical significance ($p=0.54$). Intra-individual variability was satisfactory, with $CV\%=14.7\%$ in the control group (time span= 16.7 ± 17 months), under the accepted variability threshold of 15%.

3.2 Plasma progranulin levels in *GRN* carriers: relation with gender, AAO, phenotype, disease progression and type of *GRN* mutations

We measured levels of progranulin in plasma from 160 *GRN* mutation carriers consisting of 129 FTD patients and 31 presymptomatic carriers. Median plasma progranulin levels were 36 [IQR: 29-45.7] ng/mL in the entire *GRN* carriers group. No difference was found between patients and presymptomatic *GRN* carriers ($p>0.05$) (see Table 1).

Plasma progranulin levels were significantly decreased in both *GRN* patients (36 [IQR: 29-44] ng/mL) and presymptomatic *GRN* carriers (40 [IQR: 28-54] ng/mL) with respect to controls ($p<0.001$) (see Figure 1). There was no correlation between plasma progranulin levels and the age at sampling ($rS=-0.08$, $p=0.32$). Plasma progranulin levels were significantly 10% lower in males compared to females *GRN* carriers ($p=0.019$). No statistically significant differences in plasma progranulin levels were observed according to *GRN* mutation types or localization (see Table 2).

In the group of *GRN* patients, there was no correlation between plasma progranulin levels and the age at disease onset ($rS= 0.028$, $p=0.75$, see Supplementary Figure 2). There were no significant differences between the three major FTD phenotypes *i.e.* bvFTD (n=85 patients), PPA (n=31), and CBS (n=11) (see Table 2 and Supplementary Figure 3).

3.3 The optimal threshold of plasma progranulin levels

The ROC curve analysis showed that plasma progranulin levels significantly discriminated individuals carrying *GRN* mutations from controls negative for *GRN* mutations. The area under the curve (AUC) was estimated to be 0.997 (95% confidence interval=0.993-1, $p<0.0001$) (see Figure 2A). Figure 2B shows cut-off values and the corresponding sensitivity and specificity. A cut-off of 71 ng/mL yielded the best sensitivity/specificity trade-off: 98.1% and 98.5%, respectively. Diagnostic accuracy was 98.3%.

Considering the gender difference in plasma progranulin levels, we performed distinct ROC curve analyses according to sex, and found that the optimal threshold for females (77 ng/mL, Se=96.4%, Sp=98.5%) was higher than in males (71 ng/mL, Se=100%, Sp=98.5%) (see Supplementary Tables 3 and 4).

3.4 False-negative results

Levels above the threshold of 71 ng/mL were detected on 5/270 plasmas (1.9%) of *GRN* carriers analyzed in this study (Supplementary Table 5). The five measures above the threshold were provided from 4 different *GRN* carriers. In two carriers, a second measure on an independent sample turned out to be under the threshold of 71 ng/mL (including one female whose higher level was 77 ng/mL, consistently with the cut-off established for females). Conversely, one male patient, with a family history of FTD, carrying the p.Glu498Aspfs*12 mutation had persistent normal plasma levels (85 ng/mL) at baseline and on a control sample performed 20 months later (103 ng/mL). This FTD patient suffered from morbid obesity and hyperuricemia and was treated by allopurinol and venlafaxine.

3.5 Longitudinal study of progranulin levels over time, according to disease stage and disease progression

The regression line to assess the relationship between the time from disease onset and plasma progranulin levels in all the samples obtained from *GRN* mutation carriers including presymptomatic *GRN* carriers (mean expected time to onset= -21.1 ± 11.5 years) and affected *GRN* patients (mean AAO= 59.2 ± 8.0 years, mean disease duration= 3.5 ± 3.4 years), showed overall a stability of plasma progranulin values regardless of the clinical status of carriers (see Figure 3).

In the subset of 112 participants with longitudinal plasma progranulin specimens over a mean follow-up period of 12.7 ± 11.8 months, the relative annualized change of plasma progranulin levels, from baseline to the second timepoint, was not significantly different in *GRN* carriers (n=73, relative annualized change= 4.6% [-30, +50]/year) versus controls (n=39, relative annualized change= -3.9% [-15, +26]/year, $p=0.59$).

There was also no significant difference in the relative annualized change in *GRN* carriers (n=15) (-5.8% [-7, +6.6]/year) compared to controls (n=17) (-5.8% [-7.8, 2.3]/year, $p=0.43$) when restricting the analysis to the subgroup of 32 individuals with the longest follow-up period close to four years (mean time duration between measures = 55.9 ± 7.4 months) (see Figure 4).

3.6 Influence of *TMEM106B* genotype on plasma progranulin level and age at onset

The frequency of minor G-allele was lower in *GRN* carriers compared to the European population (25% versus 40.8%, respectively) from International genome database (<https://www.internationalgenome.org/>). The frequency of GG-genotype was also lower in *GRN* carriers compared to the European population (6.1% versus 15.9%, respectively). Linear regression analyses using gender as covariate showed no significant association of rs1990622 genotypes with AAO (standardized β coefficient=0.11, $p=0.49$) and plasma progranulin levels (standardized β coefficient=0.2, $p=0.095$). Nonetheless, *GRN* carriers homozygous for the

minor G-allele had higher plasma progranulin levels (51 [36-64] ng/mL) than AA (36 [26-46] ng/mL) and AG carriers (31[24-46] ng/mL), without reaching statistical significance ($p=0.13$).

4. DISCUSSION

In this study, we evaluated plasma progranulin levels in 160 *GRN* mutation carriers consisting of 129 FTD patients and 31 presymptomatic *GRN* carriers, depicting the largest population analyzed so far. This is also the first longitudinal study evaluating progranulin plasma level changes in both controls and *GRN* carriers over a long time period (see Table 3). Our study provided evidence that plasma progranulin levels in controls did not correlate with age, and varied a little over time with a CV% of 14.7%. We confirmed that plasma progranulin levels are a valid and reliable biological marker for predicting *GRN* mutation status. This study ascertained that progranulin in plasma has an excellent screening performance, with high sensitivity and specificity for detecting *GRN* mutation carriers.

Our study estimated the optimal cut-off point to be at 71 ng/mL. There is a discrepancy in optimal cut-off values between studies (Finch et al., 2009; Ghidoni et al., 2012) that alternately established thresholds of 112 ng/mL (Finch et al., 2009), 74 ng/mL (Ghidoni et al., 2008) or 61.5 ng/mL (Galimberti et al., 2018b; Ghidoni et al., 2012) to discriminate *GRN* carriers from non-carriers (see Table 3). The most recent study suggested a cut-off of 61.5 ng/mL with a high specificity of 99.6% and a lower sensitivity of 95.8% (Ghidoni et al., 2012). Using a threshold of 61.5 ng/mL, we would have had a sensitivity of 93.8% in our population and have missed the detection of 10/160 (6.2%) *GRN* carriers. Considering the important clinical implications in routine screening settings, we rather recommend that the choice of the optimal cut-off should be based on the priority of sensitivity over specificity, to minimize false negative errors and not miss potential *GRN* mutations carriers.

As previously outlined by Nicholson et al. (Nicholson et al., 2014), plasma progranulin levels were also found to be influenced by sex in our study, with males harboring lower measures than females. We might hypothesize that the interaction of the *GRN* gene with sex steroids could play a role since it has been shown that *GRN* gene expression was induced by estrogens (Suzuki et al., 2009). This led us to stratify ROC curve analyses by gender and propose distinct thresholds, lower in males (71 ng/mL) than in females (77 ng/mL). This observation warrants further research to be confirmed with potentially important implications in clinical practice.

Our results strengthened the notion that plasma progranulin levels are decreased in presymptomatic *GRN* carriers. In our study, some carriers already showed decreased levels during their 20s, more than four decades before the expected disease onset (Galimberti et al., 2018b) (Figure 3). Our results also provided evidence for the stability of levels throughout longitudinal trajectory in *GRN* carriers. More importantly, this is the first longitudinal study demonstrating the stability of plasma progranulin levels over an extended follow-up period in *GRN* mutations carriers (>4 years in some carriers). Only one previous study showed stable levels but over a limited one-week period (Meeter et al., 2016). Our results suggest that plasma progranulin levels remain globally low, at a steady pace, throughout the natural history of disease, ranging from the presymptomatic stage to full-blown FTD, with no association with disease progression. Bringing evidence of the stability of plasma progranulin levels over long time in *GRN* mutations carriers has critical implications for the monitoring of upcoming clinical trials based on progranulin restoring therapy.

Furthermore, these findings support that plasma progranulin levels are not predictive of AAO or of clinical phenotypes. Importantly, this study is the first to provide evidence for similar levels of progranulin expression in bvFTD, PPA or CBS patients, regardless of their clinical phenotype. This suggests that the phenotypic variability of *GRN* carriers is not driven by the

level of *GRN* expression but more probably mediated by additional modifier factors. This is also consistent with the observation that serum progranulin levels only moderately correlate with its CSF levels, and thereby may not accurately reflect progranulin regulation in the brain (Meeter et al., 2016; Nicholson et al., 2014; Wilke et al., 2016). Hence, this study suggests that the pathophysiological cascade leading to focal neurodegeneration and phenotypical heterogeneity in FTD *GRN* carriers may not be fully reflected by the expression of progranulin haploinsufficiency in plasma.

Although false negative results represent only 1.9% of our series, this issue has important clinical implications. Sampling issues, technical variability or environmental factors might have transiently increased plasma progranulin levels in two carriers who had decreased levels under 71 ng/mL on follow-up samples. More strikingly, one male patient who suffered morbid obesity had normal levels at baseline as well as 20 months later. Interestingly, most other patients of this study who carried the same mutation had low plasma levels. This illustrates that progranulin expression depends not only on the presence of a given mutation, but also on external factors. For instance, dietary intake, hormonal and metabolic conditions such as insulin resistance, diabetes mellitus or obesity have been shown to increase progranulin expression (Miehle et al., 2016; Nguyen et al., 2013; Nicoletto et al., 2018; Qu et al., 2013). Morbid obesity possibly mediated higher progranulin levels in our patient, which illustrates that caution should be taken in the interpretation of plasma progranulin levels, especially in individuals harboring specific comorbidities.

Pathophysiological mechanisms implicated in *GRN*-related disorders are probably even more complex, also due to the additional role of genetic modifiers that may influence progranulin expression. The protective minor G-allele of rs1990622 in *TMEM106B* was previously associated with reduced disease penetrance in *GRN* patients (Finch et al., 2011; Lattante et al., 2014; Van Deerlin et al., 2010) and increased progranulin levels in plasma (Finch et al., 2011).

Our current results showed no association between the rs1990622 allele and plasma progranulin levels, but, among *GRN* carriers, those who were homozygous for the minor G-allele were found to have slightly higher plasma progranulin levels.

Finally, our study confirmed that progranulin expression in plasma predicts *GRN* mutation status, independently of symptoms onset proximity (Finch et al., 2009), but is not predictive of phenotype or age at onset. Nonetheless, the use of progranulin expression in plasma is a major breakthrough in the development of blood-based biomarkers of a brain-specific disorder. This work supports its value as an accurate screening test for individuals carrying *GRN* mutations, across the natural history of disease, as previously reported (Finch et al., 2009; Galimberti et al., 2018b). Furthermore, progranulin expression in plasma can be a valuable tool to support the causative role of *GRN* mutations with unknown pathogenicity (Saracino et al., 2019).

Thus, plasma progranulin levels constitute a simple, reliable and cost-effective biological marker suitable as a screening tool in clinical practice. Positive screening showing low progranulin in blood should then trigger a genetic sequencing for *GRN* mutations. An appropriate genetic counseling should be offered beforehand. The development of next generation sequencing facilitates molecular analyses of FTD genes especially in familial forms of bvFTD. However, *GRN* mutations are also responsible for 3-5% of non-familial cases (Le Ber et al., 2007), and for a larger phenotypic spectrum including PPA and CBS. These patients without family history or with an atypical presentation are less likely to be referred for molecular diagnosis and thus should primarily benefit from the progranulin plasma dosage. On the other hand, plasma progranulin dosage must not be proposed in clinical practice to individuals at risk of carrying *GRN* mutations in the context of a presymptomatic testing protocol, as their genetic status must be formally ascertained by molecular analysis. In this group, it should be limited exclusively to research settings, particularly for monitoring clinical trials based on progranulin restoring therapy.

Finally, we advocate that it could be routinely offered in clinical practice at the symptomatic stage of FTD diseases, and more broadly in the work-up of neurocognitive disorders as an aid to diagnosis. In this latter indication, further studies should be conducted to determine the diagnostic/prognostic values of plasmatic progranulin.

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Competing interests

The authors declare no conflicts of interest.

TABLES

Table 1: demographics and plasma progranulin levels in *GRN* carriers and controls

Population	<i>GRN</i> patients	Presymptomatic <i>GRN</i> carriers	Controls	<i>p</i>-value
N	129	31	133	-
Age, mean ± SD (years)	62.8 ± 7.9	39.2 ± 11.0	58.6 ± 12.6	-
Gender, female (n, %)	64 (49.6%)	19 (61.3%)	67 (50.4%)	-
Plasma progranulin levels median [IQR] (ng/ml)*	36 [29-44] ‡	40 [28-54] ‡	122 [101-158]	<0.001

Median [IQR: inter-quartile range, 25th–75th percentile], n: number, SD: standard deviation. Significant post hoc analyses are indicated by a symbol, $p < 0.05$: ‡ significant difference relative to controls, *at baseline.

Table 2: Variability of plasma progranulin levels in *GRN* mutation carriers (N=160):

Plasma progranulin levels	Median	IQR	<i>p</i> -value
Gender			
Male (n=77)	34	[28-43]	0.019
Female (n=83)	38	[31-48]	
FTD phenotypes*			
bvFTD (n=85)	35	[29-44]	0.62
PPA (n=31)	37	[30-45]	
CBS (n=11)	39	[35-43]	
<i>GRN</i> mutation type			
Frameshift (n=74)	36	[27-48]	0.44
Nonsense (n=55)	39	[30-46]	
Splice site (n=24)	35	[28-38]	
<i>GRN</i> mutation localisation			
Exon 1-6 (n=53)	35	[28-45]	0.13
Exon 9-12 (n=50)	38	[32-48]	

*In 129 patients carrying *GRN* mutations. Median [IQR: inter-quartile range, 25th–75th percentile], n: number. FTD: frontotemporal dementia; BvFTD: behavioural variant of FTD; CBS: corticobasal syndrome; PPA: primary progressive aphasia.

Table 3. Review of the studies evaluating plasma progranulin levels in *GRN* mutation carriers and controls.

	This study	Ghidoni et al., 2008	Finch et al., 2009	Ghidoni et al., 2012	Meeter et al., 2016	Galimberti et al., 2018
Total number of <i>GRN</i> carriers (n)	160	30	15	75	35	83
<i>GRN</i> patients (n)	129	8	9	49	7	19
Presymptomatic <i>GRN</i> carriers (n)	31	22	6	26	28	64
Controls (n)	133	75	70	309	29	77
Plasma progranulin threshold (ng/mL)	71.0	74.4	112.0	61.5	ND	61.5
Sensitivity (%)	99.0	100.0	100.0	95.8	ND	98.8
Specificity (%)	98.0	100.0	100.0	99.6	ND	97.4
Longitudinal analysis	Over 4 years	ND	ND	ND	Over 7 days*	ND
Elisa Kit	Adipogen	Adipogen	Adipogen	Adipogen	Biovendor	Adipogen

*18 presymptomatic carriers vs 19 controls; ND: not done

FIGURES LEGENDS

Figure 1. Box plots of plasma progranulin levels at baseline. Plasma progranulin levels were significantly lower in *GRN* carriers than *C9orf72* carriers and healthy controls.

Figure 2. ROC curve of plasma progranulin levels and sensitivity/specificity values for different cut-offs. A: the area under the curve was estimated to be 0.997, meaning that plasma progranulin levels significantly discriminated individuals carrying *GRN* mutations from controls ($p < 0.0001$). B: the proposed cut-off of 71 ng/mL yielded the best sensibility/sensitivity trade-off. ROC: Receiver-Operating characteristic.

Figure 3. Plasma progranulin levels depending on time from disease onset in *GRN* mutation carriers. The regression line (intercept = 38.04, slope = -0.05) showed overall a stability of plasma progranulin values regardless of the clinical status of carriers (n=270 values).

Figure 4. Longitudinal plasma progranulin levels in individuals with the longest follow-up period. *GRN* carriers, n=15; controls, n=17. The mean time span between the first and the last progranulin measures was 55.9 ± 7.4 months. The dash line indicates the plasma progranulin threshold (71 ng/mL).

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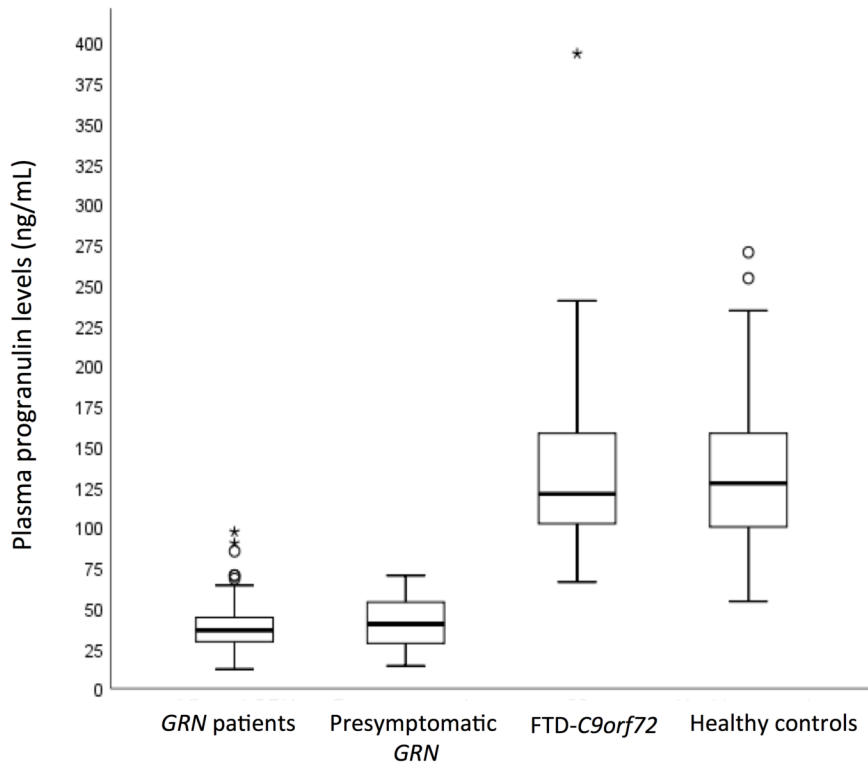
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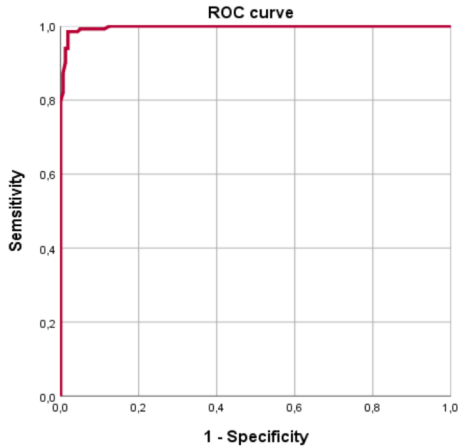
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A**B**

Cut-off values of progranulin in plasma	Sensitivity	Specificity
61.5	93.8%	99.2%
71.0	98.1%	98.5%
78.0	98.1%	96.2%
83.0	98.1%	95.5%
89.5	98.8%	90.2%
94.5	99.4%	84.2%
100.5	100.0%	75.2%
110.0	100.0%	61.7%

