

Clinical Experience with Plasma Progranulin as a Biomarker in a Dementia Cohort

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Abstract

Background: Decreased plasma progranulin levels are a specific marker in screening for Frontotemporal Lobar Degeneration (FTLD) caused by mutations in the *GRN* gene.

Objective: To describe the performance of this biomarker in clinical practice.

Methods: Using a commercial kit (AdipoGen Inc), we analysed progranulin plasma levels in 436 samples from 240 cases with FTLD phenotypes, 122 with Alzheimer type dementia, 20 with Lewy body type dementia, 15 with a psychiatric phenotype and frontotemporal atrophy, plus 39 cognitively-preserved elderly controls. The *GRN* gene was sequenced in cases with lower plasma levels. Clinical variables were correlated with plasma levels in cases with *GRN* mutations.

Results: Eight probands with FTLD phenotypes (3% of all FTLD cases, 12% of FTLD with autosomal dominant family history) and plasma levels below 70 ng/ml were found to carry seven different *GRN* mutations. The frequency of mutation-positive cases increased to 6% when considering only FTLD cases with asymmetric atrophy, though applying this criterion would have omitted two diagnosed cases. Few false positive cases (n=5) were due to technical errors, and no false negatives (70 to 90 ng/ml) were detected. In the 60 to 70 ng/ml interval, both carriers and non-carriers were found when pulling all procedures together, although they never overlapped in a single assay. None of the cases with non-FTLD phenotypes had *GRN* mutations. Plasma levels did not correlate with mutation type, age of onset, or disease stage. Asymptomatic carriers had low levels even decades before the expected age at disease onset.

Conclusion: Progranulin plasma levels are a reliable biomarker to detect the small percentage of FTLD *GRN*-mutation carriers in our cohort, though they are not useful for clinical follow-up.

Keywords: Biological markers; Frontotemporal dementia; Plasma; Progranulin levels; *GRN* mutations

Introduction

Mutations in the *GRN* gene are one of the three major genetic causes of familial Frontotemporal Lobar Degeneration (FTLD), and the only such gene with an available peripheral biomarker [1]. Physiopathologically, *GRN* mutations act through a haploinsufficiency mechanism, causing a 35% loss of functional protein, which is reflected in low levels of serum progranulin, even in individuals who are still asymptomatic [2,3-5]. Over the last decade, several studies have reported plasma and serum progranulin levels to be a reliable marker to screen for the presence of *GRN* mutations [3-8]. However, use of this biomarker remains mostly limited to research settings and it is not commonly available in routine clinical practice.

We report our experience of analyzing this biomarker on a regular basis over the past eight years (2010-2018) for clinical diagnosis of a cohort of cases with dementia. We studied well-defined FTLD phenotypes, but also a wide sample of patients presenting disease that more closely fit an Alzheimer's Type (DAT) or Lewy Body Dementia (LBD) diagnosis, but nonetheless displayed some frontotemporal features and/or had a strong family history of dementia.

Patients and Methods

This study presents data from a cohort of dementia patients recruited at the Dementia Clinic, Fundación Jiménez Díaz (Madrid, Spain) between 2006 and 2018, all of whom gave informed consent

for the study of genetic and biological markers. A majority of cases had been recruited because of a positive family history of dementia or due to presenile onset (<65 years). The population studied (n=436) is summarized in Table 1. Patients with FTLD had been diagnosed according to published standards, following the criteria of Neary et al. before 2011, and afterwards following the revised criteria of Rascovsky et al. for behavioral FTLD (FTLDbv) and the criteria published by Gorno-Tempini et al. for Primary Progressive Aphasia (PPA) [9-11]. For cases with non-FTLD diagnosis the decision was made to have the progranulin biomarker analysed based on some clinical frontotemporal features (such as predominant aphasia, predominant frontal/temporal or asymmetric atrophy), a strong family history of dementia, and/or because of early onset dementia. Control samples were from cognitively-preserved individuals, and included a subgroup of nonagenarians

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Clinical phenotype	Number of cases n= 436	Age onset, year (mean ± SD)	Plasma Pgrn levels (ng/ml)	% with APOE4	N* with GRN mutations
Frontotemporal dementia					
Non-fluent aphasia	59	65 ± 8	145,05 ± 63,58	18	4
Semantic aphasia	44	62 ± 6	169,33 ± 55,20	34	-
Behavioral variant	115	66 ± 7	149,68 ± 67,38	43	4
Behavioral variant with ALS	8	60 ± 4	136,02 ± 58,69	17	-
Progressive supranuclear palsy	9	71 ± 7	136,37 ± 62,26	33	1
Corticobasal degeneration	5	70 ± 6	165,75 ± 65,49	40	0
Non-FTD dementias					
Alzheimer type	122	61 ± 7	169,40 ± 75,24	45	-
Lewy body type	20	70 ± 7	179,26 ± 72,80	37	-
Psychiatric disease	15	63 ± 7	159,13 ± 47,17	43	-
Controls	39	85 ± 14	132,68 ± 59,12	5	-

ALS: amyotrophic lateral sclerosis. *Includes eight probands and one affected sibling of case 3

Table 1: Population studied for progranulin plasma levels.

(n=29) and ten cases with ages between 42 and 80 years. The study was approved by the hospital research ethics committee.

Plasma samples had been kept at -80°C. Progranulin levels were analysed by ELISA using a commercial kit (AdipoGen Inc, Seoul, South Korea) and following the standardized protocol. Samples were analysed in duplicate, and two positive control samples were included in every procedure. From 2010 to 2015, the commercial kit also included a quality control sample for each assay. Cases within the lower progranulin levels underwent GRN gene sequencing, which was performed by Secugen S.L. (Madrid, Spain). One family (Trp2' mutation carriers) was genetically diagnosed by whole exome sequencing conducted by the EO EUD Consortium at the VIB-UAntwerp Center for Molecular Neurology (Antwerp, Belgium), and this family has been reported recently in greater depth [12]. Plasma samples from mutation-positive cases were re-analysed all together in a single procedure to compare quantitative values.

An ANOVA test was used to compare plasma progranulin levels among FTD groups, and the Student *t* test was used to compare the FTD group with DAT, LBD, and controls. We observed relationships between plasma levels and mutation type (stop vs. missense, and among cases with the same mutation), and between clinical status (affected versus asymptomatic carriers). Finally, we searched for correlations (Pearson coefficient) between plasma progranulin levels and age at onset or years of evolution in GRN-mutation carriers, and correlations between plasma progranulin levels and age at which the sample was taken in controls.

Results

Plasma progranulin levels in all samples (except 10 outliers with levels between 350 and 450 ng/ml) are shown in Figure 1. A total of 14 ELISA analyses were conducted. We observed a wide range of values across samples, although in each individual assay there was a gap between cases with low levels which turned out to be mutation carriers and genetic-negative cases. The commercial kits maintained homogeneous accuracy and range of values over time, except for the first kit, in which the range of values was shifted significantly upwards compared to the subsequent thirteen assays.

Forty-two cases with progranulin levels <90 ng/ml underwent GRN gene sequencing. Eight cases (three with FTD bv, four with nonfluent PPA, and one with progressive supranuclear palsy) with progranulin levels ≤ 70 ng/ml were found to be carriers of seven different mutations (Table 2). Two unrelated cases carried the same M1V mutation. Six of

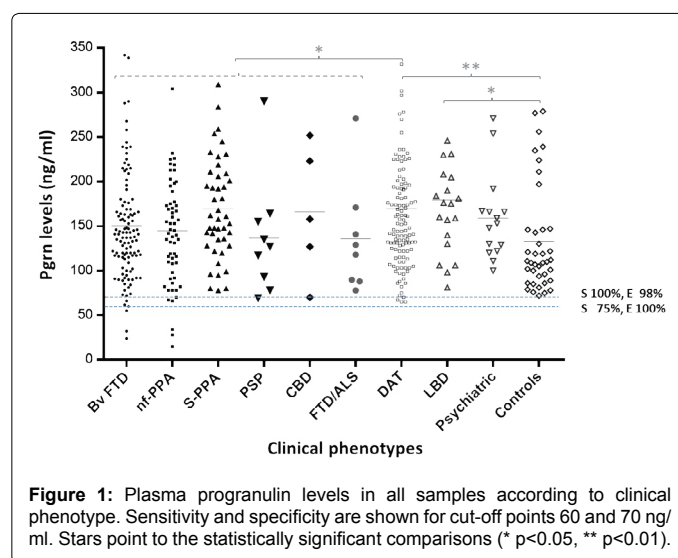


Figure 1: Plasma progranulin levels in all samples according to clinical phenotype. Sensitivity and specificity are shown for cut-off points 60 and 70 ng/ml. Stars point to the statistically significant comparisons (* p<0.05, ** p<0.01).

the GRN mutation carriers had plasma levels ≤ 60 ng/ml, while another two had progranulin levels of 67 ng/ml (case 4) and 69 ng/ml (case 6), respectively. Sequencing of the gene was negative in another five cases (three FTL D, one DAT, and one psychiatric cases) with very low levels (<30 ng/ml), but these false positive cases were due to technical errors (probably incomplete sample loading during the first procedure) because re-analyses in these samples produced levels over 100 ng/ml.

The other 29 cases (with levels >60 ng/ml) did not carry pathogenic variants. Aside from the two GRN carriers, we found nine cases with plasma progranulin levels between 61 and 70 ng/ml (five with the FTL D spectrum phenotypes and four with DAT) in which sequencing of the gene was negative. This overlap of GRN-carriers and non-carriers in the range 61 ng/ml to 70 ng/ml was only observed when pulling together all the assays, but in none of the individual procedures. We further sequenced GRN in another 20 cases with levels between 71 ng/ml and 90 ng/ml, with negative results. Cases with higher levels (>90 ng/ml) were not sequenced, as we considered it very unlikely to detect false negatives above this point. None of the cases with non-FTL D phenotypes had GRN mutations. Assuming that there were no other positive cases in the cohort (although cases with levels over 90 ng/ml were not sequenced), the 70 ng/ml cut-off point had 100% sensitivity and 98% specificity, while the 60 ng/ml threshold displayed 100% specificity though 75% sensitivity when detecting GRN carriers.

Case number c.DNA mutation NM_002087.3	Mutation protein	Age of onset/ current age (gender)	Family history*	Clinical phenotype	Parkinsonism	Atrophy on neuroimaging
1. c.1A>G	M1V	55/61^ (F)	1	Nf-PPA	Right	FT Left predom
2. c.1A>G	M1V	59/67 (F)	1	Mixed PPA	Axial	Left Fr-Parietal
3.A c.5G>A	Trp2*	65/75^ (M)	1	Bv variant	Bilateral	FT Left predom
3.B c.5G>A		50/57^ (F)		Nf-PPA	Corticobasal	FT Left predom
4. c.264+2T>C	A89VfsX41	53/59^ (M)	3.5	Nf-PPA	Corticobasal	Left perisilvian
5. c.359 C>A	S120Y	65/69 (F)	4	Semantic PPA	No	Left perisilvian
6. c.415T>C	C139R	60/? (M)	3	PSP-like	Axial	Diffuse
7. c.759_760delTG	C253X	70/77 (M)	2	Bv variant/Amnestic	Right predom	Frontal bilateral
8. c.909delC	A303AfsX57	61/? (F)	1	Bv variant	Right	FT Left predom

^Deceased; *Goldman scale: 1. Autosomal dominant. 2. Aggregation of three or more siblings 3. One affected sibling with dementia at <65 years of age. 3.5. One affected sibling with dementia after 65 years of age.

Table 2: Genetic and clinical characteristics of cases diagnosed with GRN mutations.

Identifying eight probands/families implied the genetic diagnosis of 3% of all FTLD cases, increasing to 6% when considering only FTLD cases with asymmetric atrophy (six positive cases out of 98). However, this approach would have left out two diagnosed cases. There were 33 cases with an autosomal dominant pattern of inheritance in the FTLD group, and of these, four turned out to be GRN mutation carriers (12%). The clinical characteristics of the eight probands with the seven different mutations are summarized in Table 2. All except one had a presenile age of onset (mean 59.8 years, range 51 to 70). All had a positive family history except for the carrier of the S120Y mutation. Development of parkinsonism and neuroimaging studies revealing asymmetric left predominant frontotemporal atrophy were frequent features.

Plasma progranulin levels were also analyzed in six siblings of the Trp2* mutation carrier (case 3A): one affected (case 3B), one unaffected, and four at-risk offspring. The sibling with dementia and two at-risk siblings had low progranulin levels and, as expected, they were mutation carriers. Progranulin levels in all mutation carriers (as analyzed in the same assay) are shown in Figure 2. The two mutations affecting the first amino acids were associated with the lowest plasma levels. However, there was not a clear pattern of stop mutations being associated with lower levels than missense mutations. There was no correlation between plasma levels and age at onset (R=0.03), the age at which the sample was taken (R=0.03), or years of evolution of the disease (R=0.14). In fact, the lowest levels were found in a 24-year-old asymptomatic carrier.

There were no significant differences in plasma progranulin levels across different FTLD phenotypes, or when comparing FTLD cases versus controls or LBD cases. FTLD cases had significantly lower levels

than DAT cases (p<0.05) (Figure 1). Both, DAT and LBD groups had significantly higher levels than the control group (p=0.006, and p=0.01, respectively). However, we do not support strong conclusions regarding comparisons with the control group due to their specific profile (majority of cognitively preserved nonagenarians), and because most of the control samples were analysed in a single assay, which could have biased the range of values. There was no correlation between plasma progranulin levels and age in the control group (R=0.14).

Discussion and Conclusion

As evidenced in this study, plasma progranulin levels are an efficient biomarker to detect GRN mutation carriers, although the percentage of positive cases in our FTLD cohort is small (around 3%). Our data indicate that a) analyzing cases outside of the spectrum of FTLD phenotypes is unlikely to reveal mutations in the gene; b) the number of positive cases significantly increases among patients with autosomal dominant inheritance and/or asymmetric atrophy, but limiting analysis to individuals who meet these criteria may cause positive cases to be overlooked; c) plasma levels do not correlate with mutation type or clinical variables; and d) asymptomatic carriers can have very low levels of plasma progranulin decades before the predicted age of onset of the disease. In addition, the Adipogen Elisa kit was found to be a reliable tool, exhibiting good performance in all procedures, with the most efficient cut-off point in 70 ng/ml (sensitivity 100%, specificity 98%).

In this large series we did not find GRN mutation carriers outside the FTLD spectrum phenotypes, which are in agreement with our previous experience, and with the extensive collaborative study of Yu et al. [13,14]. In the first reported series, some GRN-positive cases were found among cases with clinical diagnosis of DAT or LBD [15,16]. However, later refinement of diagnostic criteria for FTLD, together with a comprehensive characterization of GRN-mutation carrier phenotypes in several studies, has likely made clinicians better equipped to identify these cases among the FTLD spectrum [10,11,17-19]. The fact that the clinical phenotype of GRN-mutation carriers seems to be more homogeneous than the phenotypes associated with, for example, C9ORF72 expansion, facilitates this task even further [20].

The number of GRN-positive cases increased when our analysis was limited to the subgroups of FTLD cases with the autosomal dominant pattern of inheritance (12%), and to those with asymmetric atrophy (6%). Asymmetric cortical atrophy is present in about 76% of FTLD cases with GRN mutations [16,17]. In our cohort, however, only a minority of cases with FTLD and asymmetric atrophy were associated with GRN mutations. Moreover, asymmetric atrophy in the context of non-FTLD phenotypes was not associated with GRN mutations in any case, as previously reported [13].

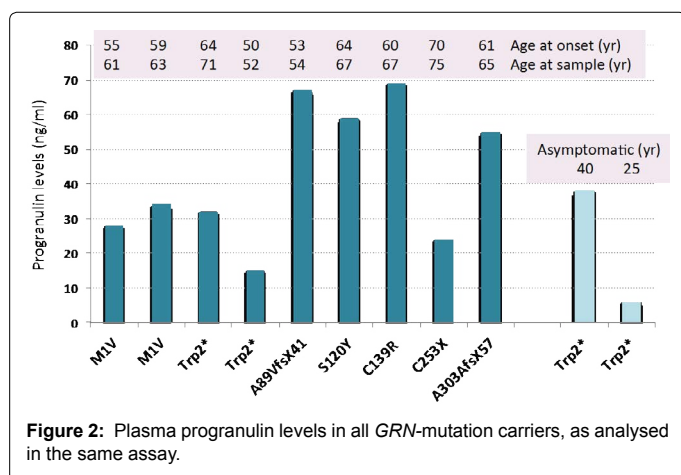


Figure 2: Plasma progranulin levels in all GRN-mutation carriers, as analysed in the same assay.

The *GRN* mutations found in our cohort are considered pathogenic, with the exception of the S120Y and C139R missense variants [12,13,21, AD & FTD Mutation Database]. The S120Y variant, currently considered as benign, has been reported in two independent European cases with ALS, though probably coming from a common ancestor [22,23]. Factors against its pathogenic impact are that Ser120 is not a highly conserved residue, the variant has also been found in one control sample, and in one carrier there were no effects on RNA or protein levels in peripheral blood [23,24]. Furthermore, ALS in not a usual phenotype associated with *GRN* mutations [18,22]. However, our S120Y carrier (case 5) has a PPA phenotype and her plasma progranulin levels were found to be consistently decreased (in two independent assays), although there was no known family history. As regards the C139R variant, which is included in the AD&FTD Mutation Database as “pathogenic nature unclear”, there are reports of families with FTLD carrying this change, as well as reported cases with a PSP phenotype resembling our case [8,25]. C139R carriers also showed low progranulin plasma levels in several studies, although the values were not as low as in cases with null mutations, suggesting a partial loss of function caused by this mutation [4,5,8,13]. Moreover, *in vitro* studies suggest that this mutation affects the function of full-length progranulin as well as elastase cleavage of progranulin into granulins, increasing susceptibility to neurodegeneration [26].

Finally, we did not find significant differences in progranulin levels by comparing different FTLD phenotypes. All *GRN*-mutation carriers had reduced levels to around one third for the mean of non-carriers, but the biomarker did not correlate with any disease measure such as clinical status, age at onset of the disease, age at which the sample was taken, or years of evolution of disease. It was clear from the family with the Trp2* mutation that asymptomatic carriers could have very low levels one to three decades before the expected age of onset. Other studies have also emphasized how asymptomatic carriers had the same reduction in progranulin levels as already symptomatic siblings [27,28]. That is, progranulin peripheral level is an efficient predictor of genetic status in population at risk but it is not a useful biomarker for clinical follow-up.

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