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Gender Effects on Plasma PGRN Levels in Patients with Alzheimer's Disease: A Preliminary Study

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Abstract. Plasma progranulin (PGRN) levels constitute a potentially invaluable biomarker for neurodegenerative diseases including frontotemporal lobar degeneration (FTLD) and, perhaps, Alzheimer's disease (AD). We assessed plasma PGRN levels in 107 AD patients, 36 FTLD patients, and 107 controls. We found that, in female AD patients, there is a positive correlation between PGRN levels and age. Although no significant differences were found between patients and controls, we observed higher levels in females compared to males; in AD patients, a positive correlation between PGRN levels and age was observed in females. In conclusion, our data suggest that PGRN may not be a good biomarker for AD; moreover, gender may influence the plasma PGRN levels of AD patients.

Keywords: Alzheimer's disease, gender, plasma, progranulin

INTRODUCTION

The neurobiology of progranulin (PGRN) has suggested that this protein may act as a neuroprotective agent against hypoxic/anoxic insults in the brain [1] and in neuroinflammation [2]. Moreover, PGRN has been found to influence the sexual differentiation of the brain; thus, in neonatal male mouse, androgen-dependent upregulation of PGRN expression in the hypothalamus is associated with its sexual differentiation [3].

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Mutations in the progranulin gene (*GRN;* MIM 138945) were identified as a causal mechanism underlying frontotemporal lobar degeneration (FTLD). Most pathogenic *GRN* mutations lead to a frameshift or premature stop codon resulting in abnormal mRNA transcripts that undergo nonsense-mediated mRNA decay resulting in a lack of expression [4, 5]. Furthermore, *GRN* mutations have been associated with widely variable clinical phenotypes, including Alzheimer's disease (AD) [6–9].

AD is the most common form of primary degenerative dementia. Clinico-pathological studies support the notion of a long 'preclinical' stage of disease; in fact it is thought that brain pathology, consisting of amyloid plaques and neurofibrillary tangles, begins 10–20

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years before significant neuronal cell death and the subsequent appearance of any cognitive and behavioral symptoms [10]. Thus, fluid and imaging biomarkers could identify subjects in early symptomatic and even preclinical stages, possibly when potential treatments can best preserve cognitive function [11].

Carriers of numerous different types of *GRN*-null mutations show significantly reduced plasma PGRN concentrations due to the loss of one functional copy of *GRN* transcript, such that PGRN levels could distinguish between carriers and non-carriers of the mutation, with a high sensitivity and specificity of roughly 100% [12]. This makes PGRN a promising biomarker to identify causative GRN mutations in patients with AD as well. However, not much is known about the possible roles of PGRN in *GRN* mutation non-carriers subjects. Recently, some common variants of *GRN* were found to be associated with AD, probably due to their influence on *GRN* mRNA expression levels [13]. In particular, the rs5848 polymorphism was found to influence serum PGRN levels, with TT carriers having a lower level of serum PGRN than CT and CC carriers [14].

In this pilot study, we assessed the possible role of plasma PGRN as a biomarker in AD, and tested whether age and gender influence its levels.

METHODS

Patients

Two hundred and fourteen subjects enrolled at the Memory Clinic, University "Sapienza", Rome and at the Alzheimer's Unit of Ospedale Maggiore Policlinico, Milan, between 2006 and 2011. The study protocol was approved by the local Institutional Review Boards and written informed consent was obtained from each participant. The control group consisted of healthy volunteers: non-consanguineous family members or caregivers of AD patients.

Patients were diagnosed according to the DSM-IV and NINCDS-ADRDA criteria [15]. All participants underwent neurological examination and standardized neuropsychological evaluation by Mini-Mental State Examination and standardized neuropsychological tests [16, 17].

One hundred and seven subjects were diagnosed with probable AD (71 women and 36 men; mean age 71.8 ± 7.8 years; duration of disease 3.80 ± 3.02 years) and the duration of disease was calculated from the onset of symptoms.

The cohort consisted of 36 patients with a familial form of AD having two or more first-degree relatives who had a history of AD-like dementia and 71 with sporadic AD. Moreover, 40 patients had an early disease onset ($\leq$65 years), whereas the remainder had a late onset (>65 years).

The control group consisted of 107 healthy volunteers matched for age, gender, and ethnic background (74 women and 33 men, mean age 69.4 ± 10.3 years). Blood samples collected into EDTA were centrifuged at 2,000 rpm for 10 min, and the plasma was collected, aliquoted, and immediately frozen at -70°C until analysis.

Genetic analysis

Genomic DNA was isolated from whole blood using a Flexigene Kit (Qiagen, Hildren, Gemany) as described by the manufacturer. To exclude the presence of causal mutations in the *GRN* gene, the entire open reading frame, including the noncoding exon 1 and exon–intron boundaries of exons 2–13 of the *GRN* gene (http://www.molgen.ua.ac.be/), was sequenced in AD patients, using specific primers [18, 19]. For APOE genotyping, DNA was amplified using specific primers and then digested with *Hha*I, as previously described [20].

Progranulin level evaluation

Plasma PGRN levels were measured using an ELISA kit (Human Progranulin ELISA Kit, AdipoGen Inc., Seoul, Korea). Samples were diluted 1:100 and processed according to the manufacturer's instructions.

Statistical analysis

The Kolmogorov-Smirnov test was applied to test for a normal distribution of plasma levels of PGRN, age, and duration of disease. Parametric tests including the *t*-test or Pearson correlation test were used to compare means in independent samples or correlate two variables when appropriate. A chi square test was used for categorical variables. A logistic-regression model was run to evaluate the relation between gender and plasma levels of PGRN adjusted for age and conditions (AD versus healthy controls). In the logistic analysis, the plasma levels of PGRN were considered in three categories according to the 33rd percentile distribution: a) low, with values included between 70 and 124.6 ng/ml; b) medium with values between 124.7

and 192.2; and c) high, with values between 192.3 and 498.6. Data were analyzed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Differences were considered to be significant at $p < 0.05$.

RESULTS

No mutations of the *GRN* gene were found in AD patients. Demographic and biochemical features of the patients and controls are summarized in Table 1.

The Kolmogorov-Smirnov test showed a statistically significant p value for the departure from normal distribution of plasma levels of PGRN ($p = 0.002$), age ($p = 0.001$), and duration of disease ($p = 0.001$). For this reason, we used parametric tests for further analyses.

No differences in age or gender distribution were observed between patients and controls ($p > 0.05$). The APOE $\varepsilon 4$ frequency was statistically different in AD patients with respect to controls (50.4% versus 16.8%; $p = 0.001$). In an overall assessment, we found no significant differences in plasma PGRN concentration between patients and healthy controls. However, a significant gender difference in plasma PGRN levels was shown, with higher levels in female (mean value ± SD: 183.91 ± 82.33 ng/ml) compared to male subjects (mean value ± SD: 152.31 ± 68.63 ng/ml; $p = 0.006$) (Table 2). In particular, the difference reached significance threshold in AD patients (females: 190.33 ± 87.40 ng/ml versus males: 154.80 ± 61.78 ng/ml; $p = 0.032$), whereas in controls, although the trend was maintained, the difference was not significant (females: 177.75 ± 77.26 versus males: 149.59 ± 76.29 ng/ml; $p = 0.083$). Moreover, we performed a sensitivity analysis excluding four extreme values of plasma levels of PGRN distribution for AD and controls. The statistical significance of comparisons did not substantially change.

In an exploratory analysis in all subjects performed on subgroups by age and gender, it appeared that PGRN levels were positively correlated with age in females ($r = 0.272$; $p = 0.001$), but not in males (Fig. 1a, b). In the AD group, this correlation was confirmed ($r = 0.252$; $p = 0.034$, Fig. 1d). Similar results were obtained in controls, in which a positive correlation between age and PGRN levels was evident in the female group ($r = 0.283$; $p = 0.015$) (Fig. 1f). The logistic regression model showed that females had a two-fold higher risk of high plasma PGRN levels (OR = 2.216; CI 95% 0.984–4.990; $p = 0.055$) than males adjusted for age and conditions (AD versus controls) (Table 2).

Finally, almost significantly different PGRN values were found between patients with a positive and negative family history of dementia (familial AD: 158.88 ± 48.37 ng/ml versus sporadic AD: 188.26 ± 92.27 $p = 0.08$). Moreover, the correlation

Table 1
Comparison between AD patients and healthy controls and between males and females

	AD	Healthy controls	p
All (numbers)	107	107	
Age at evaluation in years, mean ± SD (Range)	70.52 ± 9.26 (47–83)	69.39 ± 10.27 (43–86)	0.367
Gender (male/female)	36/71	33/74	0.660
GRN levels (ng/ml), mean ± SD (Range)	178.37 ± 81.17 (76.48–498.60)	169.06 ± 77.71 (70.35–473.40)	0.392
APOE $\varepsilon 4$ (%)	50.4	16.8	0.001
	Males mean ± SD GRN levels (ng/ml)	Females mean ± SD GRN levels (ng/ml)	
AD patients (range)	154.80 ± 61.78 (76.48–316.00)	190.32 ± 87.40 (79.60–498.60)	0.032
Healthy controls (range)	149.59 ± 76.29 (70.35–473.40)	177.74 ± 77.26 (82.02–373.40)	0.083
All subjects (range)	152.31 ± 68.63 (70.35–473.40)	183.91 ± 82.3 (79.60–498.60)	0.006

Table 2
A logistic-regression model with relation between gender (females versus males) and plasma levels of PGRN adjusted for age and conditions (AD versus healthy controls)

	B	SE	Wald	df	Sig	OR	95% CI	
							Lower	Upper
PGRN low			3.771	2	0.152	1.000		
PGRN medium	0.384	0.357	1.157	1	0.282	1.469	0.729	2.960
PGRN high	0.796	0.414	3.692	1	0.055	2.216	0.984	4.990
Age	−0.013	0.018	0.566	1	0.452	0.987	0.953	1.022
AD versus control	−0.399	0.416	0.920	1	0.338	0.671	0.297	1.516
Duration disease	0.084	0.074	1.292	1	0.256	1.088	0.941	1.259
Constant	1.317	1.225	1.156	1	0.282	3.734		

B, coefficient B; SE, standard error; Wald, Wald test; df, degrees of freedom; Sig, significance; OR, odds ratio; 95% CI, 95% confidence interval.

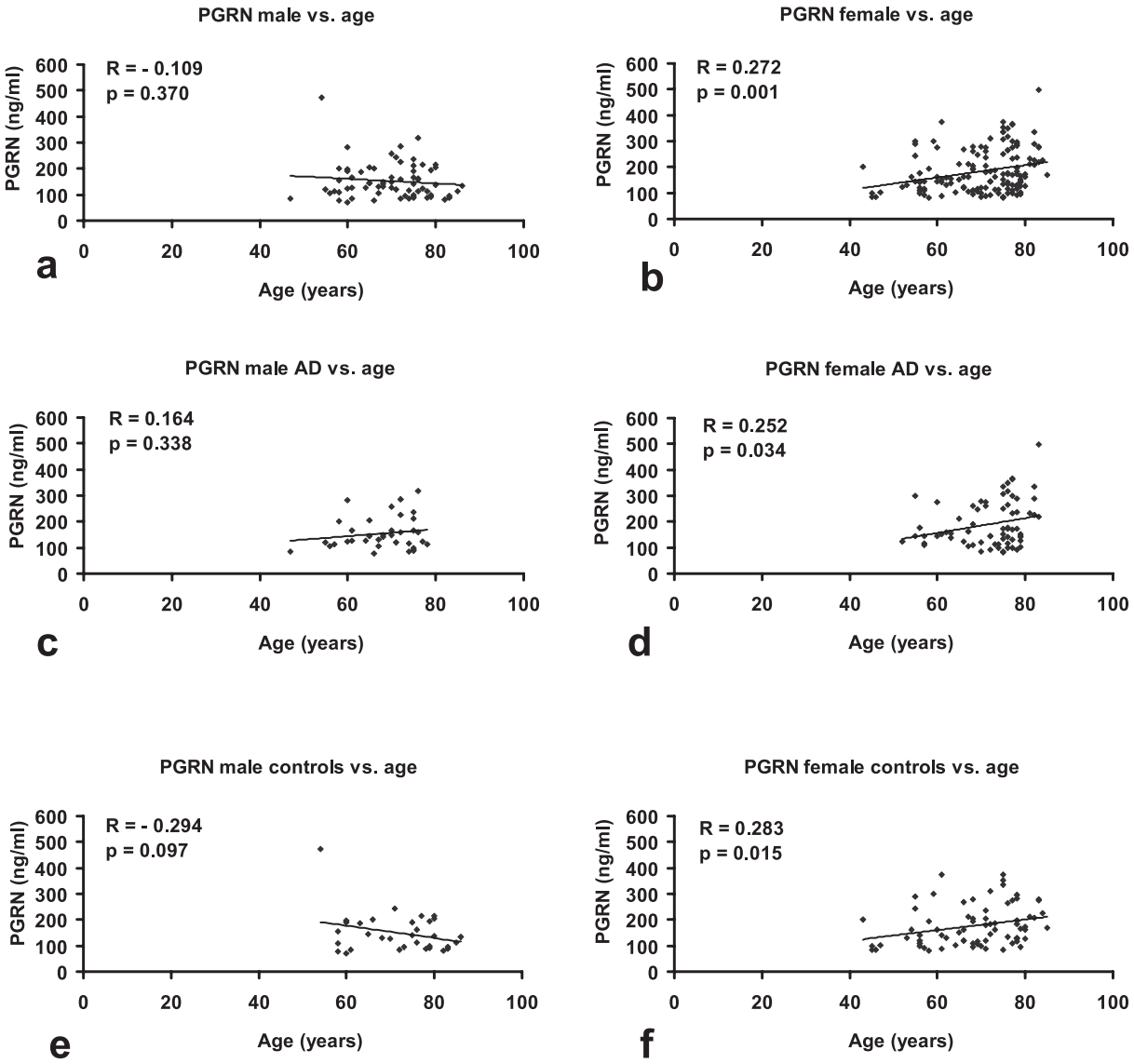

Fig. 1. Age-related increased levels on plasma PGRN are associated with gender. Correlation analysis between age and PGRN in all subjects (a, b) and in separate groups of AD (c, d) and controls (e, f).

analysis showed a borderline inverse relationship between plasmatic PGRN levels and duration of disease ($r = -0.166$; $p = 0.088$) with no difference when the analyses were performed separately for each gender.

In an ancillary analysis, performed on a small group of patients affected by FTLD (20 men and 16 women; mean age 71.3 ± 8.1 years; duration of disease 3.61 ± 2.84 years), we found a borderline gender difference in plasma PGRN levels (152.09 ± 64.15 ng/ml versus 197.74 ± 101.60 ng/ml; $p = 0.11$) and no correlation between plasmatic PGRN levels and duration of disease and age in all patients or separately by gender.

Lastly, all subjects were genotyped for APOE to determine whether the levels of PGRN were associated with the $\varepsilon 4$ allele. We found no differences between the genotype and allele frequencies of APOE and PGRN concentration.

DISCUSSION

In this study we investigated the possible differences in plasma PGRN levels between patients with AD and healthy controls. Our results showed that there were no differences between the two groups analyzed, while a significant difference was observed according

to gender, in particular in AD subjects with a higher PGRN level in females compared to males. Finch and colleagues reported a similar gender difference in the expression levels of plasma GRN in a group of FTLD patients [21].

Our data suggest a possible influence of gender on PGRN expression in some neurodegenerative diseases. In AD, female gender has been associated with an increased risk of disease development. In fact, there is general agreement on the higher prevalence of AD in women. It is well known that the main risk factor for AD is increasing age, and women have a well-known survival advantage over men [22, 23]; moreover, clear gender differences in the pathological features of AD and its relationship with behavioral disturbances indicate a biological basis for these differences [24, 25].

The dramatic loss of estrogen at menopause is generally acknowledged as a risk factor for the development of AD in women [26–28]. Although there is no consensus on differences in circulating levels of estradiol in control subjects and women with AD, brain estrogen have been shown to be lower than normal in female subjects with AD [29, 30]. In the last decade, a novel biological aspect of PGRN as a mediator in sexual differentiation of the developing brain was demonstrated such that PGRN may be involved in masculinization of the perinatal rat brain. In fact, transcription of PGRN was upregulated by exogenous estrogen in the neonatal hypothalamus [31] and, in males, it is maintained at high levels throughout the critical period, while in females it gradually decreases and then declines abruptly after birth [32]. Moreover, in adult rats PGRN gene expression is upregulated by estrogen in the hippocampus [33]. These data suggest a role of PGRN in sexual differentiation of the developing brain and an influence of estrogen on its expression.

Regarding the PGRN expression in patients with AD and healthy controls, we found no differences between these two groups, but we observed an inverse relationship between plasmatic PGRN levels and duration of disease. Our results do not seem to support a major role of PGRN in AD as a possible biomarker in plasma samples. However, it could potentially be used as a marker of disease progression. Moreover, when the analyses were performed separately for gender, we found a weak but not significant difference between AD and controls in females. Interestingly, the gender-dependent differences in PGRN expression were more evident in affected females compared to healthy subjects; this inhomogeneity could be associated with a different effect of PGRN on disease between women and men. Gender-dependent differences in AD could also be very effective in determining drug efficacy in AD therapy. It is well known that men have a 73% greater chance of responding to anticholinesterase therapies than women [34].

However, on the basis of the number of male and female AD patients and the observed mean and standard deviation of PGRN plasmatic values in the two groups, we can estimate a power of 69.3% in these findings.

In conclusion, we observed an association between the female gender and PGRN levels, as well as a correlation between such levels and age in the female population, both with and without AD. Further studies in a larger population would be needed to confirm the gender-related involvement of PGRN in the pathogenesis of AD and in the different forms of disease, and to better characterize its role in disease progression.

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