Low Serum Progranulin Predicts the Presence of Mutations: A Prospective Study

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Abstract. Serum progranulin is decreased in frontotemporal dementia (FTD) patients with progranulin gene (PGRN) mutations. We investigate the utility of prospective serum screening as a surrogate diagnostic marker for progranulin mutations. A commercial ELISA was used to measure progranulin protein concentration in serum from 63 FTD patients and 32 normal controls, and DNA screening then performed. Four patients (2/17 behavioral variant, 2/8 corticobasal syndrome) had abnormally low progranulin levels with PGRN mutations confirmed on DNA testing. Surprisingly, elevated levels were found in 6/16 patients with progressive non-fluent aphasia, the significance of which is unclear. Serum testing is an accurate and cost effective means of predicting PGRN mutations.

Keywords: Enzyme-linked immunosorbent assay, frontotemporal dementia, GRN protein, hematologic tests, human, mutation

INTRODUCTION

Progranulin (PGRN) gene mutations have been found in approximately 8% of frontotemporal dementia (FTD) patients [1] and are associated with reduced brain and serum levels of progranulin [2]. Mutations are most common in patients with behavioral variant FTD (bvFTD), and least common in semantic dementia (SD) and FTD with motor neuron disease (FTD-MND) [1]. PGRN null-mutations are associated with an approximate four-fold reduction in blood plasma progranulin levels relative to controls [2]. A smaller reduction in serum progranulin is also related to missense mutations [3] whilst benign polymorphisms caused no change in serum levels. Studies to date have compared patient groups with mutations to those without [4], rather than to normal controls. Genetic screening is expensive and of limited availability, especially in the developing world. We investigated the diagnostic utility of serum progranulin levels as a surrogate marker for PGRN mutations in a prospective study of FTD patients of all subtypes.

METHODS

Serum and DNA samples were collected from consecutive patients with FTD attending a specialist FTD clinic (FRONTIER) in Sydney, Australia. Written informed consent was obtained for all participants in this study and the study was approved by the Human Research Ethics Committees of the South Eastern Sydney and Illawarra Health Service-Northern Hospital Network, and the University of New South Wales. All patients underwent a full neurological and cognitive assessment by an experienced neurologist (JRH). Care-
**Table 1**

<table>
<thead>
<tr>
<th>Disease group</th>
<th>n</th>
<th>Sex</th>
<th>Age ± SD</th>
<th>Serum PGN (ng/ml)</th>
<th>Pathologically low serum levels*</th>
<th>Patients with high serum levels*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>32</td>
<td>15:17</td>
<td>66 ± 9</td>
<td>165 ± 42 (75–242)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>bvFTD</td>
<td>17</td>
<td>7:10</td>
<td>60 ± 9</td>
<td>120 ± 34 (37–164)</td>
<td>2 (12%)</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>11</td>
<td>4:7</td>
<td>60 ± 7</td>
<td>134 ± 31 (106–215)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PNFA</td>
<td>16</td>
<td>8:8</td>
<td>69 ± 11</td>
<td>201 ± 77 (109–395)</td>
<td>0</td>
<td>6 (38%)</td>
</tr>
<tr>
<td>FTD-MND</td>
<td>11</td>
<td>2:9</td>
<td>63 ± 9</td>
<td>156 ± 64 (114–338)</td>
<td>0</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>CBS</td>
<td>8</td>
<td>4:4</td>
<td>63 ± 8</td>
<td>127 ± 54 (29–176)</td>
<td>2 (25%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Greater than 2.5 standard deviations from mean control serum progranulin levels.

**RESULTS**

The range of control values (Table 1) was very similar to that reported [2,3]. When all FTD subtypes were considered together in comparison to controls, there was a significant reduction in the mean levels of serum progranulin of 15 ng/ml (Mann Whitney U, \( p = 0.017 \), Fig. 1). Serum progranulin levels were abnormally low (< 2.5 standard deviations from mean control level) in 6%. In addition, seven subjects were found to have second degree relatives with another neurodegenerative condition.
### Table 2

<table>
<thead>
<tr>
<th># in Fig. 1</th>
<th>Disorder</th>
<th>Age</th>
<th>Sex</th>
<th>Serum progranulin ng/ml</th>
<th>Ethnicity</th>
<th>Family history</th>
<th>Mutation&lt;sup&gt;b&lt;/sup&gt; (predicted protein)</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CBS</td>
<td>57</td>
<td>M</td>
<td>29 (3.2 SD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Swiss</td>
<td>two paternal uncles with dementia</td>
<td>exon 9 g.11315_11316insGT = rs63751239 (p.Trp304fx)</td>
<td>Yes [9]</td>
<td></td>
</tr>
<tr>
<td>2. bvFTD</td>
<td>58</td>
<td>M</td>
<td>37 (3.0 SD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dutch-Australian</td>
<td>father non-AD dementia, died aged 75</td>
<td>exon 7 g.10577delT (p.Ser203fx)</td>
<td>Yes [7]</td>
<td></td>
</tr>
<tr>
<td>3. bvFTD</td>
<td>53</td>
<td>F</td>
<td>44 (2.8 SD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>British</td>
<td>father with “Pick’s” (pathology FTD-U) paternal uncle with dementia paternal aunt with “Pick’s”&lt;sup&gt;c&lt;/sup&gt;</td>
<td>exon 2 g.9132_9133insCTGC (p.Cys31fx)</td>
<td>Yes [10]</td>
<td></td>
</tr>
<tr>
<td>4. CBS</td>
<td>54</td>
<td>F</td>
<td>58 (2.5 SD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lebanese</td>
<td>cousin with CBS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>exon 9 g.11303C&gt;T (p.Gln300X)</td>
<td>Yes [7]</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of standard deviations below mean control serum progranulin.

<sup>b</sup>Mutation annotated relative to nucleotide position number 1 in Genebank genomic sequence NG_007886.1.

<sup>c</sup>Segregation of mutation observed within pedigree.

abnormally high levels of serum progranulin, the majority with PNFA (6 subjects) and one with FTD-MND (Table 1).

Screening for PGRN mutations was undertaken in the four FTD subjects with abnormally low serum progranulin levels (Fig. 1). All four had young onset FTD (ages 53 to 58) and were ethnicity diverse; two had bvFTD and two CBS. All four patients were found to have nucleotide substitutions, or insertions/deletions in the coding sequence of the PGRN gene that are predicted to result in truncated proteins (Table 2). Three of these nucleotide changes were known pathogenic mutations, whilst patient 2 had a novel nucleotide deletion (g.10577delT) in exon 7 that is predicted to give rise to a frame-shift at codon 203 (p.Ser203fx) in the PGRN transcript. Of interest, identical frameshift mutations in the PGRN transcript (p.Ser203fx), arising from different nucleotide deletions, have been reported in other FTLD pedigrees [7]. Only two reported an autosomal dominant pattern of inheritance, the other had a weaker family history which was only later uncovered by further genealogical analysis. We were able to demonstrate segregation of the g.9132_9133insCTGC in a daughter and her father, and the g.11303C>T PGRN mutation in two affected cousins.

**DISCUSSION**

This is the first study to prospectively confirm that serum progranulin protein levels can identify FTD patients with null mutations in the PGRN gene. In the four patients with at least a 2.5 standard deviation depression of serum progranulin, there was 100% accuracy in determining PGRN abnormalities. The identification of mutations in bvFTD and CBS subtypes, and not in language subtypes of FTD, is in line with the reported incidence of PGRN mutations [1,6]. An interesting additional finding was that abnormally high levels of progranulin protein were detected in patients with PNFA (38% of subjects), whilst patients with SD, all had serum progranulin levels within the normal range.

In one retrospective study, 94 ng/ml was measured for null mutations although those with pathogenic missense mutations had higher levels that overlapped with healthy controls [4]. Another study of progranulin protein in plasma identified 74.4 ng/ml as a cutoff [2], a level more in line with our findings. We propose that a serum progranulin level of 67 ng/ml might be an optimal cutoff below which a PGRN mutation would be highly likely. We also note that there was no correlation between the level of serum progranulin protein and position of the PGRN mutation, as mutations in exon 9 resulted in the lowest and highest serum progranulin levels within the group of four PGRN mutation carriers.

There is no clear hypothesis for the increased levels of progranulin in PNFA which is typically associated with tau pathology [8], although it suggests that levels of progranulin protein may be modulated by other pathological variations.

In conclusion, given the expense of genetic screening and the difficulties sometimes in establishing a relevant
family history, the clinical utility of serum progranulin screening in FTD patients is considerable.

ACKNOWLEDGMENTS

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REFERENCES