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Progranulin mutation analysis: Identification of one novel mutation in exon 12 associated with frontotemporal dementia

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Abstract

Progranulin (*PGRN*) mutations account for an average of 15% of familial FTD cases and 20% of total FTD cases worldwide. Here we investigated the frequency of *PGRN* mutations in FTD patients (n=116) from a clinical cohort of South India and detected one novel mutation located on exon 12 in a familial bvFTD patient (accounting for ~1% of total FTD cases and 6% of familial FTD cases). This mutation was found to introduce a premature termination codon and the prematurely terminated mRNA may probably undergo nonsense-mediated decay. In ELISA, the proband showed significantly reduced level of plasma progranulin (28 ng/mL) compared with controls (150±38 ng/mL), which implicates haploinsufficiency as the pathogenic mechanism.

Keywords

Frontotemporal dementia; Progranulin; PGRN; Null Mutation; Nonsense-mediated decay

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1. Introduction

Frontotemporal dementia (FTD) represents the second most prevalent cause of presenile dementia after Alzheimer's disease (AD). The most common presentation of this debilitating disorder is as a sporadic disorder; however the familial incidence has been reported in up to 50% of cases. There are mainly three genes identified that are shown to play a causal role in the familial cases. These are microtubule-associated protein tau (*MAPT*), progranulin (*PGRN*) and chromosome 9 open reading frame 72 (*C9ORF72*) (Baker, et al., 2006, Cruts, et al., 2006, DeJesus-Hernandez, et al., 2011, Hutton, et al., 1998, Renton, et al., 2011). Among them, human *PGRN* encodes a widely expressed growth factor and has been shown to be mutated in ~15% of the familial cases and some proportion of sporadic cases. Null mutations in *PGRN* were shown to cause FTD in several independent studies (Gijssels, et al., 2008). So far there are no studies addressing the role of *PGRN* mutations in FTD from South India. Through this study, we analyzed the frequency of *PGRN* mutations in a clinically confirmed cohort of 116 FTD cases.

2. Materials and Methods

Study participants were recruited from patients attending the Memory and Neurobehavioral Clinic at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) between 2008 and 2012, after obtaining approval from the Institutional Ethical Committee of SCTIMST and written informed consent from all of the participants or their caregivers. These patients were evaluated with longitudinal clinical, neuropsychological and neuroimaging assessments and diagnosed as FTD as per the consensus criteria (Neary, et al., 1998). The study cohort comprised patients diagnosed with behavioral variant FTD (bvFTD) (n=94), progressive nonfluent aphasia (PNFA) (n=12), semantic dementia (SD) (n=7), FTD associated with Amyotrophic lateral sclerosis (FTD-ALS) (n=3) and cognitively unimpaired controls (n=130) who were matched with the patients for age and ethnicity. None of the patients carried any known genetic mutations in *MAPT* (Aswathy, et al., 2014). Mutation analysis was performed through direct sequencing of entire coding sequence (exons 1 to 12 and non-coding exon 0) (Cruts, et al., 2006). Plasma concentrations of progranulin were determined using a solid-phase sandwich ELISA kit (Human PGRN ELISA Kit, Adipogen Inc., Seoul, Korea) in a total of 60 FTD samples (mean age $\pm$ SD: 64 $\pm$ 9 years) and 36 age-matched controls (mean age $\pm$ SD: 63 $\pm$ 7 years). Statistical analysis was performed using GraphPad Prism software 5.01 (San Diego, CA, USA).

3. Results and Discussion

This is the first study to determine the prevalence of *PGRN* mutations in a South Indian FTD cohort. The FTD group comprised 71 males and 45 females with mean age of 63 $\pm$ 10 years and mean age of onset as 61 $\pm$ 9 years. A positive family history was noticed in 15% FTD cases (17/116). Extensive mutation analysis has identified one novel heterozygous mutation in a familial bvFTD patient. The mutation was found to be a C>T transition that occurred in exon 12 of *PGRN* at position +94th relative to the first coding nucleotide of exon 12 (Figure 1). The functional check was done with PROVEAN and found that this mutation introduces a premature termination codon at codon 503 coding for the amino acid glutamine (p.Gln.

503X) and would be predicted to terminate the mRNA prematurely, likely leading to the partial loss of functional progranulin through nonsense-mediated decay as it was established for several other truncating *PGRN* mutations (Baker, et al., 2006, Cruts, et al., 2006, Gass, et al., 2006). This mutation was absent in the remaining unrelated patients and controls ruling out the possibility of a founder mutation. The proband was a 76 year old woman with a positive familial history for cognitive impairment diagnosed with bvFTD at the age of 75 years. The *PGRN* expression analysis was carried out in a subset of patients and controls in which the plasma samples were available. Even though the comparison did not reveal any statistical significance, in proband the concentration was 28 ng/mL, which is reduced up to about one third with respect to non-mutation carriers and controls (cut off value: 112 ng/mL as proposed by Finch, et al., 2009) indicating haploinsufficiency as the pathogenic mechanism. The overall frequency of *PGRN* mutation was found to be ~1% (1/116) in total FTD cases and 6% (1/17) in FTD with a positive family history which is much lower when compared with the study by Gass et al (about 10% of total FTD and 23% of familial FTD cases) (Gass, et al., 2006). This discrepancy is likely explained by the fact that the previous reports estimated the frequency of *PGRN* mutations in pathologically confirmed cases with ubiquitin positive inclusions, whereas the current study did not include such patients. In addition to this novel mutation, the sequencing of *PGRN* in FTD samples identified eight sequence variants. Of these, three were novel non-synonymous changes occurring within the intron 1 and exon 11. These SNPs and their frequencies in FTD are listed in the Table 1. The pathologic significance of the novel SNPs influencing *PGRN* expression was analyzed through ELISA and showed normal progranulin levels. We conclude that known genetic mutations in *PGRN* play little role in the etiology of FTD patients from South India and the presence of novel variants suggest that the population may be peculiar in its phenotypic expression and when studying their genetics novel variants and novel disease mechanisms should be addressed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Frontotemporal dementia (FTD) is a devastating clinical syndrome associated with the progressive degeneration of frontal and anterior temporal lobes of the brain which is specifically involved in the behavior control, decision-making, emotions and language. Up to 50% of FTD cases possess a family history of similar dementia and in remaining cases the disease is sporadic in nature. This is the first report to analyze the genetic mutations in PGRN in the current cohort. The whole PGRN gene was screened for mutations and identified one novel mutation in exon 12 with the proposed uniform disease mechanism of haploinsufficiency.

Figure 1.
Electropherogram showing p.Gln.503X mutation in PGRN exon 12 (C to T).

Table 1
Variants identified in *PGRN* and their frequency in FTD identified through this study

Intron/Exon	Chromosome position	Genomic variation	Protein change	rs ID	Frequency in FTD
IVS 1-41		T>C	Nil	Novel	8%
IVS 1-29		G>A	Nil	Novel	15%
IVS 3+21		G>A	Nil	rs9897526	42%
IVS 5+24		G>A	Nil	rs850713	50%
Exon 5	g.1098	T>C	Asp128	rs25646	21%
IVS 9-36	g.2350	C>T	Nil		3%
Exon 11		G>C	Leu469Phe	Novel	6%
Exon 12		C>T	Gln503X	Novel	6%
5'-UTR		C>T	Nil	rs5848	47%

Two SNPs in intron 1 such as IVS1-41, IVS1-29 and one exonic variant in exon 11 are novel non-pathogenic polymorphisms identified in this study. Gln503X is the novel mutation identified in this study. All other SNPs detected were previously reported and are non-pathogenic.