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Research Paper

Association Between Progranulin and Gaucher Disease

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ABSTRACT

Background: Gaucher disease (GD) is a genetic disease caused by mutations in the *GBA1* gene which result in reduced enzymatic activity of β-glucocerebrosidase (GCase). This study identified the progranulin (PGRN) gene (*GRN*) as another gene associated with GD.
Methods: Serum levels of PGRN were measured from 115 GD patients and 99 healthy controls, whole *GRN* gene from 40 GD patients was sequenced, and the genotyping of 4 SNPs identified in GD patients was performed in 161 GD and 142 healthy control samples. Development of GD in PGRN-deficient mice was characterized, and the therapeutic effect of rPGRN on GD analyzed.
Findings: Serum PGRN levels were significantly lower in GD patients (96.65 ± 53.45 ng/ml) than those in healthy controls of the general population (164.99 ± 43.16 ng/ml, $p < 0.0001$) and of Ashkenazi Jews (150.64 ± 33.99 ng/ml, $p < 0.0001$). Four *GRN* gene SNPs, including rs4792937, rs78403836, rs850713, and rs5848, and three point mutations, were identified in a full-length *GRN* gene sequencing in 40 GD patients. Large scale SNP genotyping in 161 GD and 142 healthy controls was conducted and the four SNP sites have significantly higher frequency in GD patients. In addition, "aged" and challenged adult PGRN null mice develop GD-like phenotypes, including typical Gaucher-like cells in lung, spleen, and bone marrow. Moreover, lysosomes in PGRN KO mice exhibit a tubular-like appearance. PGRN is required for the lysosomal appearance of GCase and its deficiency leads to GCase accumulation in the cytoplasm. More importantly, recombinant PGRN is therapeutic in various animal models of GD and human fibroblasts from GD patients.
Interpretation: Our data demonstrates an unknown association between PGRN and GD and identifies PGRN as an essential factor for GCase's lysosomal localization. These findings not only provide new insight into the pathogenesis of GD, but may also have implications for diagnosis and alternative targeted therapies for GD.

1. Introduction

Gaucher disease (GD), a common lysosomal storage disease (LSD), is caused by mutations in *GBA1* with resultant defective glucocerebrosidase (GCase) function and the consequent accumulation of its substrate glucosylceramide (β-GlcCer) in macrophages and other cell types (Platt, 2014). There are three types of GD based on its

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neurological complications (type 1 is non-neuropathic, type 2 is acute neuropathic and type 3 is chronic neuropathic). Extra-neurologic systematic features include hepatosplenomegaly, pancytopenia, and osteoporosis as a consequence of Gaucher cell infiltration in target organs. GD has been regarded as wholly attributable to *GBA1* mutations. However, clinical manifestations may have huge variations among patients carrying the same *GBA1* mutations, ranging from very early disease onset to very mild clinical presentations (Biegstraaten et al., 2011; Elstein et al., 2010). It has therefore been speculated that additional disease modifiers exist in GD patients.

Progranulin (PGRN), also known as granulin epithelin precursor (GEP), is recognized for its roles in a variety of physiologic and disease processes, including immunomodulation (Jian et al., 2013a), cell growth, wound healing (He and Bateman, 2003), host defense (Park et al., 2011) and inflammation (Park et al., 2011; Tang et al., 2011; He et al., 2003). PGRN acts as an anti-inflammation molecule by direct binding to TNF receptors (Tang et al., 2011; Jian et al., 2013b). PGRN also functions as an important neurotrophic factor and mutations of the *GRN* gene (coding PGRN) are directly linked to frontotemporal dementia (Baker et al., 2006; Cruts et al., 2006), as well as considered contributory to other neurological diseases (Mateo et al., 2013; Perry et al., 2013). PGRN has been shown to play an important role in lysosomes, and homozygous mutation of the *GRN* gene results in neuronal ceroid lipofuscinosis (Smith et al., 2012; Gotzl et al., 2014). In this study we reported PGRN as a novel disease modifier in GD. In addition, recombinant PGRN is therapeutic against GD in various preclinical models.

2. Methods

2.1. Study Participants

Serum samples from 115 GD with N370S mutation in *GBA1*, 44 healthy controls from the general population and 55 healthy controls from Ashkenazi Jews were collected from New York University Medical Center and Beth Israel Medical Center (Fig. 1a). Genomic DNA samples from 161 GD and 142 healthy controls from GP were collected in New York University Medical Center. Serum levels of PGRN were measured and whole *GRN* gene was sequenced in 40 GD patients, and SNP genotyping was performed in 161 GD and 142 GP control DNA samples. All of patients have signed informed consent forms. This study is approved by the IRB institute of New York University School of Medicine. All samples were stored in −80 °C.

2.2. Serum Levels of PGRN

Serum levels of PGRN were measured by ELISA kit from Adipogen (Cat. No. AG-45A-0018TP-KI01, San Diego, CA). Briefly, the ELISA plated were blocked with 300 μl blocking buffer for 30 min. During that time, sera were diluted 200 fold by PBS. The blocking buffer was discarded and 100 μl samples were loaded as well standards (starting from 4 ng/ml to 0 ng/ml) for 2 h. Plates were washed with PBS 5 times and 100 μl Detection Antibody was added for 1 h. Plates were washed again and 100 μl Detector was added for another hour. Plates were rinsed and 100 μl TMB Substrate Solution was added and the reaction was terminated by Stop solution. The results were readout at 450 nm using a plate reader. The concentrations of PGRN were calculated based on the standard curve. The serum progranulin cutoff level is 61.55 ng/ml based on literature (Ghidoni et al., 2012).

2.3. Amplification of GRN Gene

Genomic DNA was isolated from peripheral blood cells of GD patients using DNA Purification kits from QIAGEN. 40 Genomic samples were randomly chosen, and were used as templates to amplify the whole *GRN* gene, including the 1 kb promoter region and the 8 kb full-length *GRN* gene. Five pairs of primers were designed to cover the 9-kb *GRN* gene with certain overlap between two adjoining fragments. A barcode sequence was added to 5′-end of all five pairs of *GRN* gene primers, and each patient has a unique barcode sequence and shared the same *GRN* gene specific primers. A total of 200 (40 × 5) primers were synthesized (Supplementary Table S1). *GRN* gene was amplified by Phusion® High-Fidelity DNA Polymerases (NEB Inc., Ipswich, MA). All 200 PCR products were mixed at an equal molar ratio into one tube. This final sample was sent to Genomic facility of Yale University for sequencing.

2.4. Sequencing of GRN Gene

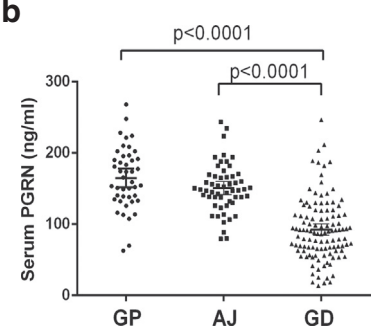
A novel technology, PacBio RS II Sequencing System, was used to sequence 40 samples at one time (Supplementary data Fig. S1a) (Eid et al.,

a

Demographic information of human samples

	GP Control	AJ control	GD patients
Gender (% men)	54.5%	50.9%	51.5%
Age (age ±SD)	50.2±11	48.8±15	51.9±20
Case No.	44	55	115

b



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	N	Mean	S. D.	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Healthy Controls of GP	44	164.99	43.16	151.87	178.11	62.92	268.16
Healthy Controls of AJ	55	150.64	33.99	141.45	159.83	79.35	243.43
Gaucher's Disease (GD)	115	96.65	53.45	86.78	106.53	13.71	263.85
Total	214	124.58	55.96	117.04	132.12	13.71	268.16

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	198913.150	2	99456.575	44.822	<0.0001
Within Groups	468192.757	211	2218.923		
Total	667105.907	213			

Fig. 1. GD patients have decreased levels of PGRN. (a) Demographic information of human subjects. (b, c) Serum levels of PGRN from 115 GD patients, 44 healthy controls from GP, and AJ were measured by ELISA. GD patients have significantly lower levels of PGRN (96.65 ± 53.45 ng/ml) than healthy controls from GP (164.99 ± 43.16 ng/ml), and AJ healthy controls (150.64 ± 33.9 ng/ml), $p < 0.0001$.

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