Parkinsonism and Related Disorders 20 (2014) 1215–1220

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Glucocerebrosidase mutations in primary parkinsonism

Rosanna Asselta [a], Valeria Rimoldi [a], Chiara Siri [b], Roberto Cilia [b], Ilaria Guella [a], Silvana Tesei [b], Giulia Soldà [a], Gianni Pezzoli [b], Stefano Duga [a, **], Stefano Goldwurm [b, *]

[a] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milano, Italy
[b] Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, Italy

ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form
26 August 2014
Accepted 1 September 2014

Keywords:
Parkinson's disease
GBA
Parkinsonism
Association analysis
Splicing mutation
Functional characterization

ABSTRACT

Introduction: Mutations in the lysosomal glucocerebrosidase (*GBA*) gene increase the risk of Parkinson's Disease (PD). We determined the frequency and relative risk of major *GBA* mutations in a large series of Italian patients with primary parkinsonism.

Methods: We studied 2766 unrelated consecutive patients with clinical diagnosis of primary degenerative parkinsonism (including 2350 PD), and 1111 controls. The entire cohort was screened for mutations in *GBA* exons 9 and 10, covering approximately 70% of mutations, including the two most frequent defects, p.N370S and p.L444P.

Results: Four known mutations were identified in heterozygous state: 3 missense mutations (p.N370S, p.L444P, and p.D443N), and the splicing mutation IVS10+1G>T, which results in the in-frame exon-10 skipping. Molecular characterization of 2 additional rare variants, potentially interfering with splicing, suggested a neutral effect. *GBA* mutations were more frequent in PD (4.5%, RR = 7.2, CI = 3.3–15.3) and in Dementia with Lewy Bodies (DLB) (13.8%, RR = 21.9, CI = 6.8–70.7) than in controls (0.63%). but not in the other forms of parkinsonism such as Progressive Supranuclear Palsy (PSP, 2%), and Corticobasal Degeneration (CBD, 0%). Considering only the PD group, *GBA*-carriers were younger at onset (52 ± 10 *vs.* 57 ± 10 years, $P < 0.0001$) and were more likely to have a positive family history of PD (34% *vs.* 20%, $P < 0.001$).

Conclusion: *GBA* dysfunction is relevant for synucleinopathies, such as PD and DLB, except for MSA, in which pathology involves oligodendrocytes, and the tauopathies PSP and CBD. The risk of developing DLB is three-fold higher than PD, suggesting a more aggressive phenotype.

1. Introduction

Mutations in the gene encoding beta-glucocerebrosidase (*GBA*, OMIM *606463) are an important and common risk factor for Parkinson's disease (PD). Many studies have shown an increased frequency of *GBA* mutations in PD compared to controls [1,2]. In particular, an odds ratio (OR) of approximately 5 has been found in a multicenter study including approximately 5000 PD patients and an equal number of controls [3]. *GBA* mutations were repeatedly found to be increased also in Dementia with Lewy Bodies (DLB) [4–6]. A recent multicenter study on 700 DLB patients reported a remarkable OR of 8, suggesting that *GBA* mutations may have an even larger role in the genetic etiology of DLB than in PD [7]. Furthermore, PD carriers of the *GBA* mutation are more likely to progress to dementia, suggesting a significant impact on the distribution of pathology and on the resulting clinical phenotype [8]. On the other hand, Multiple System Atrophy (MSA) does not appear to be associated with *GBA* [9,10]. So far, no data are available on *GBA* involvement in tauopathies, such as Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD).

The frequency and distribution of *GBA* mutations vary among populations, hindering comparisons between different patient series. Carrier frequency is quite high among Ashkenazi Jews (about 1 person in 14), and very rare in Asia [3]. In addition, studies were usually performed on series of patients with the same diagnosis often collected from many clinical centers, preventing the evaluation of the importance of *GBA* in the different forms of parkinsonism.

In this frame, we decided to analyze the major mutations in the *GBA* gene in a large series of unrelated patients with primary

* Corresponding author. Parkinson Institute, Istituti Clinici di Perfezionamento, via Bignami 1, 20126 Milano, Italy. Tel.: +39 02 5799 3319; fax: +39 02 5799 3468.
** Corresponding author. Department of Medical Biotechnology and Translational Medicine, University of Milan, Via Viotti 3/5, 20133 Milan, Italy. Tel.: +39 02 50315823; fax: +39 02 50315864.
E-mail addresses: stefano.duga@unimi.it (S. Duga), goldwurm@parkinson.it, stefano.goldwurm@gmail.com (S. Goldwurm).

http://dx.doi.org/10.1016/j.parkreldis.2014.09.003

parkinsonism visited in a single Italian tertiary clinic and to compare their frequency with a large control group from the same population. This design enabled the evaluation and comparison of *GBA* burden in various forms of parkinsonism in the Italian population.

2. Methods

This study was approved by the local Ethical Committee and was conducted according to the Declaration of Helsinki and to the Italian legislation on sensitive personal data recording. Written informed consent was obtained from all subjects.

2.1. Subjects

We studied 2766 unrelated consecutive patients with degenerative parkinsonism, and 1111 controls, who contributed from 2002 to 2010 to the Parkinson Institute Biobank (www.parkinsonbiobank.com), regardless of family history or age at onset. All the patients had a diagnosis of primary degenerative parkinsonism: 2350 fulfilled current criteria for probable PD, 29 for DLB, 118 for MSA, 100 for PSP, and 34 for CBD [11,12]. In particular, diagnosis of DLB was made only in those fulfilling the 1-year rule between the onset of dementia and parkinsonism [13]. In the remaining 135 cases, the clinical diagnosis was still uncertain and these patients are reported here as suffering from undefined primary parkinsonism (PKS). The majority of MSA cases ($N = 113$) had been previously reported in a multicentre collaborative study on *GBA* involvement in MSA [10]. Patients with suspect of secondary parkinsonism were excluded. All patients were examined by neurologists expert in movement disorders. The following clinical and demographic data were collected: gender, age at onset, asymmetry of symptoms at onset, disease duration, education, cigarette smoking, and family history of PD. Among the 2350 PD patients, the mean age at onset was 56.1 years (SD ± 10.9, range 13–87), the mean disease duration was 11.6 years (SD ± 6.7, range 5–56).

Controls were recruited among spouses and caregivers and were unrelated to the patients. All subjects who reported or showed signs or symptoms of movement disorders or other neurodegenerative diseases were excluded. Among the 1111 controls, the mean age at sample collection was 62.3 years (SD ± 11; range 30–94 years). All controls denied any family history for movement disorders in first-degree relatives.

Except for 25 patients originating mainly from other European countries, all patients and controls were of Caucasian ethnicity and Italian origin.

2.2. Mutation analysis

The mutational screening of *GBA* exons 9 and 10 was performed by a combination of high-resolution melting (HRM) analysis (exon 9) and direct DNA sequencing (exon 10). PCR primer couples were designed on the basis of the known genomic sequence of the gene (GenBank accession number NM_000157) to amplify the two exons of interest and their exon-intron boundaries, avoiding the concomitant amplification of the highly-homologous *GBA* pseudogene (*GBAP1*) (Supplementary Table 1). A detailed description of mutation analysis methods is reported in the Supplementary material.

The 2350 PD patients were previously tested for several PD-related genes, such as *LRRK2* (G2019S, R1441 C/G, I2020L), *Parkin*, *PINK1*, *DJ1*, and *SNCA* [14–16]. The 66 patients found to be carrier of mutations in these genes were not excluded from the *GBA* genetic analysis.

2.3. Molecular characterization of the newly-identified splicing mutation

The effect of the IVS10+1G>T splicing mutation on *GBA* pre-mRNA processing was evaluated in RNA derived from whole blood of the carrier patient. Whole blood was collected in a PAXgene Blood RNA Tube (PreAnalytiX, Hombrechtikon, Switzerland) and RNA purification performed by using the PAXgene Blood miRNA kit (PreAnalytiX) following the manufacturer's instructions. One microgram of total RNA was reverse transcribed (RT) using random nonamers and the Superscript-III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Of a total of 20 μL, 1 μL was used as template for standard PCR reactions by means of exonic primers (Supplementary Table 1). The identity of the amplified fragments was confirmed by Sanger sequencing. To quantify the relative amount of *GBA* exon-10 containing *vs.* skipping isoforms, we performed competitive RT-PCRs by using a 6-FAM-labeled primer. Amplified fragments were separated by capillary electrophoresis on an ABI-3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and quantitated by the GeneMapper v4.0 software.

2.4. Statistical analysis

All the following statistical procedures were performed using the R program release 2.8.0 (http://www.r-project.org/).

For each *GBA* mutation, standard case–control analyses on carrier frequency data were performed with the Fisher exact test; all *P* values are presented as non-corrected. The impact of *GBA* mutation burden on different clinical subtypes was evaluated by the use of the relative risk (RR) statistics.

The association of different variables with the presence of *GBA* mutations in PD patients was assessed by testing for differences between carriers and non-carriers by means of a chi-square test for categorical data (i.e. gender, asymmetric onset, smoking status, family history of PD), or by using the Student's *t* test for continuous data (i.e. age at onset, disease duration, education; these were analyzed as a quantitative variable, after having verified that their departure from linearity was not statistically significant). The effect of each factor was expressed as the OR and 95% confidence interval (CI). Unadjusted ORs were obtained by using a logistic regression model that included only the factor of interest; adjusted ORs were obtained by using a model that included the factor of interest plus all of the factors that were significant in the first step of analysis.

3. Results

3.1. Screening for GBA mutations on exons 9 and 10

In the whole cohort of subjects investigated (2766 patients and 1111 controls), we identified 10 different rare genetic variants, all present in the heterozygous state (Table 1).

The p.N370S and p.L444P mutations were more common in patients than in controls (2.5% *vs.* 0.36%, $P = 1.2 \times 10^{-6}$ OR = 7.1, CI = 2.6–19.5; and 1.7% *vs.* 0.27%, $P = 1.1 \times 10^{-4}$ OR = 6.4, CI = 2.0–20.6, respectively) (Supplementary Table 2). In addition to

Table 1
GBA mutations/rare variants identified in 2766 patients with degenerative parkinsonism and 1111 healthy controls.

Genomic position[a]	Rs ID	cDNA change[b]	Function[c]	Alleles in cases (*n*)	Alleles in controls (*n*)
1:155,205,659	NA	c.1225-24T>G	IVS8-24T>G	0	1
1:155,205,634	rs76763715	c.1226A>G	p.N370S[d]	69	4
1:155,205,581	rs149171124	c.1279G>A	p.E388K	5	1
1:155,205,440	NA	c.1388 + 32C>T	IVS9+32C>T	1	0
1:155,205,138	NA	c.1389-36C>G	IVS9−36C>G	0	1
1:155,205,107	NA	c.1389-5T>A	IVS9-5T>A	1	0
1:155,205,047	rs75671029	c.1444G>A	p.D443N[d]	1	0
1:155,205,043	rs421016	c.1448T>C	p.L444P[d]	47	3
1:155,204,985	NA	c.1505G>T	IVS10+1G>T[d]	1	0
1:155,204,978	rs371668537	c.1505C>A	IVS10+8C>A	1	0

All variants were identified in the heterozygous state. Frequent polymorphisms (minor allele frequencies >2.5%) are not reported.
Seventeen of the 47 L444P mutations found in cases and two of the 3 found in controls were associated with the p.A456P variant (indicating the presence of a complex recombinant allele).
Rs ID, refseq identification number; NA, not available [variant not reported either in the HGMD professional, or in 5400 exomes obtained from the Exome Variant Server, NHLBI GO exome Sequencing Project (ESP, v.0.0.9 data release, November 2011, http://evs.gs.washington.edu/EVS/)].
[a] According to UCSC Genome Browser (http://genome.ucsc.edu/, release Feb. 2009; GRCh37/hg19 assembly).
[b] According to mRNA Accession# NM_000157.3.
[c] Protein numbering omitting the signal peptide.
[d] Mutations already known to be responsible for Gaucher Disease.