Glucocerebrosidase Mutations in a French-Canadian Parkinson's Disease Cohort

Anne Noreau, Jean-Baptiste Rivière, Sabrina Diab, Patrick A. Dion, Michel Panisset, Valérie Soland, Nicolas Jodoin, Mélanie Langlois, Sylvain Chouinard, Nicolas Dupré, Guy A. Rouleau

Can. J. Neurol. Sci. 2011; 38: 772-773

Gaucher disease is a rare Mendelian disorder characterized by mutations in lysosomal enzyme glucocerebrosidase (GBA) gene, leading to accumulation of the glycolipid glucosylceramide. At the present time, over 335 mutations have been reported in GBA, most of which are predicted to lead to loss of enzyme function (Human Gene Mutation Database, HGMD http://www.hgmd.cf. ac.uk/ac/index/.php). Gaucher Disease is divided into three types, based on the clinical symptoms and neurological manifestations: type 1 non-neuronopathic (MIM#230800); type 2 acute neuronopathic (MIM#230900) and type 3 subacute neuronopathic (MIM#231000); type 2 and 3 are the most severe forms. Recently, family studies showed an increased incidence of Parkinson's disease (PD) in relatives of patients with Gaucher disease, suggesting that haploinsufficiency of GBA may predispose to PD[1]. A multicenter analysis of GBA reported a higher frequency of missenses mutations in PD patients than in healthy control individuals[1]. Interestingly, this result was even more significant when only individuals of Jewish descent were considered; 15% of PD patients in this subgroup had either an L144P or a N370S mutation, which is five times higher than what can be observed in healthy controls of Jewish descent. In non-Jewish individuals the mutation frequency in PD patients was 3% which is significantly higher than in matched healthy controls, where it was less than 1%. This observation was subsequently replicated in a PD sample of Chinese origin, but not replicated in a North-African Berber-Arab Tunisian population of PD cases[2,3]. The goal of our study was to evaluate the possible involvement of the GBA mutations in a French-Canadian PD cohort.

Methods

Polymerase chain reaction (PCR) Amplifications and Sequencing

Using 11 sets of primers the entire coding region of GBA was sequenced and primers were designed to allow preferential amplification of GBA (NM_001005749) over the GBA pseudogene located 16 kb upstream on the same chromosome. Polymerase chain reactions were performed using the AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, California, USA) as per manufacturer's instructions. Polymerase chain reaction products were sequenced at the Genome Quebec Innovation Centre (Montréal, Québec, Canada) using a 3730XL DNAnalyzer (Applied Biosystems, Foster City, California).

Selection and Description of Participants

French-Canadian PD patients from two movement disorders clinics were enrolled in the study after they gave informed consent. The study was approved by the local ethics review boards (Centre Hospitalier de l'Université de Montréal and Centre Hospitalier Affilié Universitaire de Québec). Parkinson's disease subjects were recruited on the basis of their symptoms such as resting tremor, bradykinesia/akinesia and postural reflex impairment. The selected patients also met the Ward and Gibb criteria for PD[4]. All PD subjects were levodopa responsive, further supporting the clinical diagnosis of PD. Our cohort contained 212 French-Canadian patients with a clear PD diagnosis and 189 healthy age-matched French-Canadian controls. Mainly composed of 71% males (n=151) with mean age of onset of 56.7 years old (range 30-80), the cohort largely contained sporadic PD cases since only 8.5% of cases had an affected relative. The age-matched French-Canadian control individuals were also seen by a neurologist specialised in movement disorders and confirmed to be healthy. Control individuals were recruited over the years; they are mostly spouses or unaffected siblings of patients seen in Tourette Syndrom, Restless leg syndrom or Parkinson clinics. For this study we selected subjects that were over 50-years-old, French-Canadian and confirmed to be healthy by a neurologist. This combination of criteria decreased the possibility of having subjects who could develop PD and ensured ethnical similarities. Genomic DNA was extracted from blood samples using the Puregene DNA kit and according to the manufacturer's protocol (Gentra System, USA).

Analysis and Statistics

Mutation surveyor (v.3.10, SoftGenetics, State College, Pennsylvania) was used for mutation detection analysis. All

From the Center of Excellence in Neuroscience of Université de Montréal (CENUM), Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) (AN, JBR, SD, PAD, SC, GAR); Department of Pathology and Cellular Biology (PAD), Faculty of Medicine, Department of Medicine (GAR), University of Montreal; André-Barbeau Movement Disorders Unit (MP, VS, NJ, SC), University of Montreal Health Centre (CHUM); Research Center (GAR), CHU Sainte-Justine, Montreal, Quebec; Faculty of Medicine (ML, ND), Laval University, Department of Neurological Sciences, CHA-Enfant-Jésus, Quebec, Quebec, Canada.

Received January 6, 2011. Final Revisions Submitted March 17, 2011.
Correspondence to: Guy A. Rouleau, CHUM Research Centre, 2099, Alexandre De-Seve Street, Room Y-3633, Montreal, Quebec, H2L 2W5, Canada.

Table 1: Non-synonymous coding variants in cases vs. controls

Cases (212)	Controls (189)	Variant*	Variant	Polyphen score	dbSNP
1	0	p.L236F	p.L197F	1.837	
5	3	p.E365K	p.E326K	0.698	rs2230288
1	0	p.S378L	p.S339L	1.518	
9	5	p.T408M	p.T369M	1.409	rs2230289
0	2	p.N409S	p.N370S	1.703	
1	0	p.W417G	p.W378G	4.141	
5	1	p.L483P	p.L444P	2.043	rs35095275
22	**11**				

* nomenclature with 39 peptide-signal amino-acids.

variations were confirmed by re-amplifying the PCR fragment and a second resequencing. Mutation frequencies in cases and controls were compared using Fisher's exact test, where P<0.05 was considered statistically significant.

Results

An overall sequencing success rate of 99.5% was obtained for PD cases and controls; the confirmed non-synonymous coding variants are shown in Table 1; variants are indicated using the two known nomenclatures for GBA (with or without the presence of the 39 amino acids from the signal peptide). Considering all sequence variants, a frequency of 10% is seen in PD cases, compared to a frequency of 6% in control individuals; this difference is not significant (Fisher p value 0.1048, Odd ratio (OR) 1.87). Certain variants do not have a neuronopathic role in Gaucher Disease (p.T408M, p.E365K and p.N370S; Human Gene Mutation Database). Moreover, the variant p.E365K retains about 50% of enzymatic activity and is associated with a mild phenotype, while the p.N370S is always associated with a mild phenotype with low penetrance, presumably because it is far from the catalytic site of GBA enzyme[5]. The remaining variants, L444P along with three novel variations p.L236F, p.S378L and p.W417G are predicted to be damaging using the bioinformatic software Polyphen (Table 2). Of these probably pathological variants, eight are found in 212 PD cases and only one in 189 controls, thus the mutation frequencies are 3.77% for PD cases and 0.5% for controls; a significant differences according to the Fisher's Exact test (p=0.0397, OR 7.35).

Table 2: Pathogenic mutations in GBA cases vs controls

Variant *	Cases (212)	Controls (189)	Polyphen Score
p.L236F	1	0	1.837
p.S378L	1	0	1.518
p.W417G	1	0	4.141
p.L483P	5	1	2.043
total	**8**	**1**	

* nomenclature with 39 peptide-signal amino-acids.

Discussion

Our study indicates that GBA variations likely contribute to the development of PD in French-Canadians. The mutation frequency found for the possibly pathogenic mutation is 3,77%, that is almost the same rate found by Sidransky et al, for all the populations combined[1]. None of our PD cases with a pathogenic mutation had a familial history of PD or Gaucher Disease. In fact, the probability that these PD patients had a relative with Gaucher disease is really low, mainly because they are all sporadic PD cases and also because Gaucher disease is rare in the French-Canadian population. In the province of Quebec, there are only 30 patients that are followed and 15 are treated for this disorder. These 30 cases harboured mainly the treatable Gaucher type I. Gaucher disease type II is more rare with incidence less than 1/500 000 birth. The fact that the p.L444P variation is the most frequently observed GBA mutation in the French-Canadian population is consistent with our results. For the three novel variations, it is possible these mutations are specific to the French-Canadian population and/or that the changes are embryonic lethal when homozygous, hence they have not yet been found in Gaucher disease patients. Functional analysis will be needed to determine if these variations are truly pathogenic or whether they are polymorphisms having little impact on the role of the enzyme.

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https://doi.org/10.1017/S0317167100012300 Published online by Cambridge University Press