ORIGINAL ARTICLE

Mutations in the Glucocerebrosidase Gene and Parkinson's Disease in Ashkenazi Jews

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ABSTRACT

BACKGROUND

A clinical association has been reported between type 1 Gaucher's disease, which is caused by a glucocerebrosidase deficiency owing to mutations in the glucocerebrosidase gene (*GBA*), and parkinsonism. We examined whether mutations in the *GBA* gene are relevant to idiopathic Parkinson's disease.

METHODS

A clinic-based case series of 99 Ashkenazi patients with idiopathic Parkinson's disease, 74 Ashkenazi patients with Alzheimer's disease, and 1543 healthy Ashkenazi Jews who underwent testing to identify heterozygosity for certain recessive diseases were screened for the six *GBA* mutations (N370S, L444P, 84GG, IVS+1, V394L, and R496H) that are most common among Ashkenazi Jews.

RESULTS

Thirty-one patients with Parkinson's disease (31.3 percent; 95 percent confidence interval, 22.2 to 40.4 percent) had one or two mutant *GBA* alleles: 23 were heterozygous for N370S, 4 were heterozygous for 84GG, 3 were homozygous for N370S, and 1 was heterozygous for R496H. Among the 74 patients with Alzheimer's disease, 3 were identified as carriers of Gaucher's disease (4.1 percent; 95 percent confidence interval, 0.0 to 8.5 percent): 2 were heterozygous for N370S, and 1 was heterozygous for 84GG. Ninety-five carriers of Gaucher's disease were identified among the 1543 control subjects (6.2 percent; 95 percent confidence interval, 5.0 to 7.4 percent): 92 were heterozygous for N370S, and 3 were heterozygous for 84GG. Patients with Parkinson's disease had significantly greater odds of being carriers of Gaucher's disease than did patients with Alzheimer's disease (odds ratio, 10.8; 95 percent confidence interval, 3.0 to 46.6; $P<0.001$) or control subjects (odds ratio, 7.0; 95 percent confidence interval, 4.2 to 11.4; $P<0.001$). Among the patients with Parkinson's disease, patients who were carriers of Gaucher's disease were younger than those who were not carriers (mean [±SD] age at onset, 60.0±14.2 years vs. 64.2±11.7 years; $P=0.04$).

CONCLUSIONS

Our results suggest that heterozygosity for a *GBA* mutation may predispose Ashkenazi Jews to Parkinson's disease.

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PARKINSON'S DISEASE IS A COMMON neurodegenerative condition, with an estimated prevalence of approximately 1 in 100 persons.[1] Parkinson's disease is characterized by resting tremor, akinesia, rigidity, and postural instability, caused by selective degeneration of dopaminergic neurons within the substantia nigra pars compacta and consequent depletion of dopamine in their striatal projections.[1] Most cases of Parkinson's disease are sporadic, and familial cases are rare.[2,3] Data from twin and family studies,[4] the mapping and cloning of *PARK* genes, and analysis of potential susceptibility genes have provided increasing evidence to indicate a causative role for genetic factors in the disease.[5,6] There is also evidence indicating that environmental factors play a role in the causation of Parkinson's disease.[7] The association of parkinsonism with type 1 Gaucher's disease has been reported.[8-12] The simultaneous occurrence of Parkinson's disease and Gaucher's disease is marked by atypical parkinsonism generally presenting by the fourth through sixth decades of life. The combination progresses inexorably and is refractory to conventional antiparkinson therapy.[11]

Gaucher's disease, the most prevalent, recessively inherited disorder of glycolipid storage,[13] is caused by a deficiency of the lysosomal enzyme glucocerebrosidase, which normally hydrolyzes glucocerebroside to glucose and ceramide, leading to the accumulation of glucocerebroside in macrophages and resulting in multiorgan involvement.[13] Three phenotypes have been described that are denoted by the absence (type 1) or presence of neurologic involvement during childhood (type 2) or adolescence (type 3).[13] Type 1 Gaucher's disease is panethnic, but is especially prevalent among persons of Ashkenazi Jewish descent, with a carrier rate of 1 in 17 Ashkenazi Jews.[14] The N370S and 84GG mutations are the most frequent mutations in the glucocerebrosidase gene (*GBA*) among Ashkenazi Jews, with rates of 1 in 17.5 for N370S and 1 in 400 for 84GG in the general healthy Ashkenazi population, and are associated with mild and severe Gaucher's disease, respectively. The 84GG mutation occurs almost exclusively among Ashkenazi Jews.[14] Other rare *GBA* variants identified in patients of Ashkenazi descent with Gaucher's disease include L444P, IVS2+1G→A, V394L, and R496H.

In an attempt to establish whether there is an association between Parkinson's disease and Gaucher's disease, we determined the prevalence of mutations in the *GBA* gene in 99 Ashkenazi patients with idiopathic Parkinson's disease, who had no signs or symptoms of Gaucher's disease, and compared the rate with that among Ashkenazi patients with Alzheimer's disease and among healthy Ashkenazi controls.

METHODS

POPULATION

Ninety-nine Ashkenazi patients with idiopathic Parkinson's disease (55 men and 44 women) were sequentially recruited from the Cognitive and Movement Disorder Unit at the Rambam Medical Center, Haifa, Israel, on their arrival at the clinic for follow-up or treatment over a period of 28 months (from February 21, 2002, to April 30, 2004). None had a history of neurologic or psychiatric conditions other than Parkinson's disease. Seventy-four patients with Alzheimer's disease (42 men and 32 women) were similarly recruited from the same clinic to serve as a comparison group. The clinic serves as a secondary and tertiary referral center for patients with Alzheimer's disease and Parkinson's disease from the northern part of Israel. Parkinson's disease was diagnosed according to the United Kingdom brain-bank criteria.[15] Patients with Alzheimer's disease met the criteria for dementia of the Alzheimer's type of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition,[16] and the criteria for probable Alzheimer's disease of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.[17]

Information on family history, demographic characteristics, and clinical data were obtained in a uniform manner with the use of structured questionnaires. Patients underwent a physical, neurobehavioral, and neurologic examination that incorporated the Unified Parkinson's Disease Rating Scale.[18] Patients or their guardians were asked to provide written informed consent, and patients were asked to provide a blood sample. All patients were informed regarding the results of the analysis. The study was approved by the hospital's institutional review board. A control group of 1543 healthy Ashkenazi Jews from the same geographic area who were undergoing testing to identify heterozygosity for certain recessive diseases and who provided written informed consent allowing the use of their DNA for research purposes was used to determine the frequency of *GBA* mutations in our general population.

DETECTION OF MUTATIONS

DNA samples were subjected to a polymerase-chain-reaction (PCR) assay to identify six *GBA* mutations (N370S, L444P, 84GG, IVS2+1G→A, V394L, and R496H). PCR amplification was followed by digestion with appropriate enzymes (Table 1), to distinguish the wild-type allele from the mutant allele.[19] Six primer pairs were used separately to amplify the genomic segments flanking each mutation. The PCR primers, annealing temperatures, restriction enzymes, and length of the PCR products before and after cleavage are listed in Table 1. The L444P and R496H mutations create cleavage sites with the use of the *Nci*I and *Hph*I restriction enzymes, respectively. The IVS2+1G→A mutation abolishes a native restriction site for *Hph*I. A mismatch introduced in either the forward or reverse primer is used to create a restriction site in either the mutant PCR product (N370S and 84GG) or the normal PCR product (V394L) with the use of *Xho*I for N370S, *Bsab*I for 84GG, and *Ban*I for V394L. All mutant-allele profiles were confirmed by means of sequence analysis in an independent PCR assay, with the use of an automated ABI Prism 310 Genetic Analyzer (Perkin–Elmer Applied Biosystems). No discrepancies were detected between the results of cleavage analyses and the results of sequencing (Fig. 1 and 2).

STATISTICAL ANALYSIS

Differences in carrier rates among groups were analyzed by means of the chi-square test. Differences in clinical characteristics were compared between carriers and noncarriers by means of an independent-sample t-test for age and a chi-square test for family history.

RESULTS

Among the 99 Ashkenazi patients with Parkinson's disease, 31 (31.3 percent; 95 percent confidence interval, 22.2 to 40.4 percent) had a mutant *GBA* allele (Table 2): 23 were heterozygous for N370S, 3 were homozygous for N370S, 1 was heterozygous for R496H, and 4 were heterozygous for 84GG. Among the 74 patients with Alzheimer's disease, 3 were carriers of Gaucher's disease (4.1 percent; 95 percent confidence interval, 0.0 to 8.5 percent); 2 were heterozygous for N370S, and 1 was heterozygous for 84GG. Among the 1543 control subjects, 95 were carriers of Gaucher's disease (6.2 percent; 95 percent confidence interval, 5.0 to 7.4 percent); 92 were heterozygous for N370S, and 3 were heterozygous for 84GG, findings consistent with a carrier rate of 1 in 16.7 for the N370S variant and 1 in 514 for 84GG. Patients with Parkinson's disease had significantly greater odds of being carriers of Gaucher's disease than did patients with Alzheimer's disease (odds ratio, 10.8; 95 percent confidence interval, 3.0 to 46.6; P<0.001) or control subjects (odds ratio, 7.0; 95 percent confidence interval, 4.2 to 11.4; P<0.001). The rate of carriage of Gaucher's disease among patients with Alzheimer's disease did not differ significantly from that

Table 1. Primers and Variables Used for the Detection of Mutations in the *GBA* Gene.

Mutation	Primer Sequence*		Annealing Temperature	Restriction Enzyme	Size of Fragment		
	Forward	Reverse	°C		PCR	Wild Type (*base pairs*)	Mutant (*base pairs*)
A1226G (N370S)	GCCTTTGTCCTTACCCTC†G	GACAAAGTTACGCACCCAA	56	*Xho*I	105	105	89, 16
84GG	GAATGTCCCAAGCCTTTGA	CACTGCCTGAAGTAGA†GC	57	*Bsab*I	75	75	56
IVS2+1G→A	GAATGTCCCAAGCCTTTGA	AAGCTGAAGCAAGAGAATCG	57	*Hph*I	358	141, 117, 100	241, 100
G1297T (V394L)	ACCGACTGGAACCTTGCCCTG	GACTGTCGACAAAGTTAG†GC	57	*Ban*I	69	69	47, 22
T1448C (L444P)	GGAGGACCCAATTGGGTGCGT	ACGCTGTCTTCAGCCCACTTC	56	*Nci*I	638	638	536, 102
G1604A (R496H)	TCCTGGAGACAATCTCACCT	AAGCTCACACTGGCCCTGC	56	*Hph*I	160	160	114, 36

* Primer sequences are listed from the 5' end to the 3' end.
† A mismatch was introduced in the primer at one nucleotide to create a restriction site.

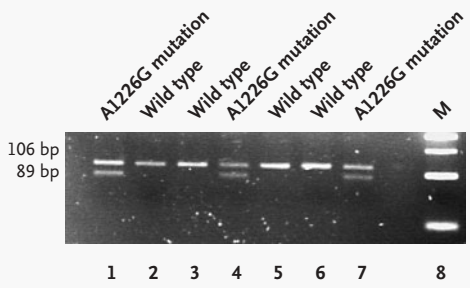

Figure 1. PCR Analysis of the A1226G (N370S) Mutation in Patients with Parkinson's Disease.

When the mutation is present (lanes 1, 4, and 7), the enzyme (*Xho*I) digests a 105-bp PCR product, producing two fragments of 89 and 16 bp. The wild-type PCR product remains uncut (lanes 2, 3, 5, and 6). M denotes a 50-bp marker.

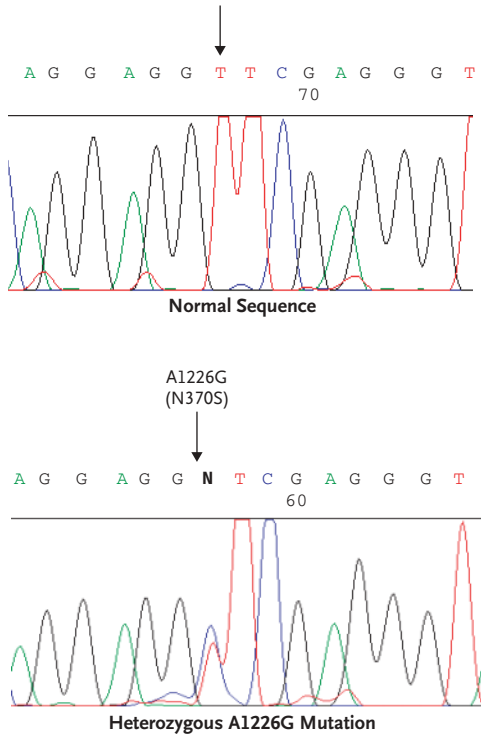

Figure 2. Electropherogram of the Normal Sequence and the A1226G (N370S) Mutation in the *GBA* Gene.

The arrows show the position of the mutation.

among controls (odds ratio, 0.6; 95 percent confidence interval, 0.2 to 2.2; P=0.62).

All patients with Parkinson's disease had an initially favorable response to dopaminergic agonists or levodopa. Among the patients with Parkinson's disease, those who were also carriers of Gaucher's disease were significantly younger than those who were not carriers (mean [±SD] age at onset, 60.0±14.2 years vs. 64.2±11.7 years; P=0.04). Carriers of Gaucher's disease did not differ significantly from noncarriers with regard to the presence of a family history of Parkinson's disease in a first- or second-degree relative, initial motor manifestations, or initial response to levodopa or dopaminergic agonists.

DISCUSSION

Because parkinsonism has occasionally been described in patients with Gaucher's disease, we evaluated the effect of *GBA* mutations on idiopathic Parkinson's disease. In our population of patients with Parkinson's disease, the frequency of a mutant N370S *GBA* allele was 5 times that among our healthy Ashkenazi control subjects, and the frequency of a mutant 84GG *GBA* allele was 21 times that among controls (P<0.001 for both comparisons). In addition, three patients with Parkinson's disease were found to be homozygous for nonpenetrant Gaucher's disease (N370S/N370S), as compared with none of the 1543 control subjects. Since N370S causes a mild phenotype, N370S/N370S homozygotes may remain symptom-free, and their Gaucher's disease may escape detection. The prevalence of *GBA* mutations in our population of Ashkenazi patients with Parkinson's disease by far outweighs the reported prevalence of mutations in other susceptibility genes for Parkinson's disease, such as parkin and synuclein.[20] Mutations in the *GBA* gene thus emerge as strong genetic determinants predisposing people to Parkinson's disease.

The nature of the association between Parkinson's disease and Gaucher's disease remains elusive. In recent years, two hypotheses regarding the pathogenesis of Parkinson's disease have been suggested. The first posits that misfolding and aggregation of proteins are instrumental in the death of dopaminergic neurons, and the other proposes that the culprit is oxidative stress resulting from mitochondrial dysfunction, which may also increase the amount of misfolded proteins.[21] The aggregation of proteins may lead to cell dysfunction by inhibiting the ubiquitin–proteasome system,[22,23] a finding that has been implicated in the causation of both familial and sporadic Parkinson's disease.[24]

We speculate that the pathogenic mechanism leading to Parkinson's disease in carriers of mutant

Table 2. Rates of Carriage of Gaucher's Disease among Patients with Parkinson's Disease, Patients with Alzheimer's Disease, and Control Subjects.

Population	No. Tested	No. of Carriers (%)	95% Confidence Interval
Patients with Parkinson's disease	99	31 (31.3)	22.2–40.4
Patients with Alzheimer's disease	74	3 (4.1)	0.0–8.5
Controls	1543	95 (6.2)	5.0–7.4

GBA alleles may be related to the faulty processing of toxic, unwanted proteins, aggravated by the relative decrease in glucocerebrosidase activity and accumulation of glucocerebroside. Indeed, studies demonstrate that the inhibition of glucocerebrosidase and accumulation of glucocerebroside induce apoptosis in cultured neurons by increasing the mobilization of calcium ions from intracellular stores[25] and that neurons with elevated levels of glucocerebroside show enhanced sensitivity to agents that induce cell death by potentiating calcium ions.[26] Moreover, mesencephalic cells, including dopaminergic neurons, can undergo apoptosis after ceramide-induced damage,[27] suggesting that dysfunctional metabolism of sphyngolipids may induce the death of dopaminergic cells. However, since brain glucocerebroside levels were not consistently elevated in patients with type 1 Gaucher's disease,[28] the pathogenetic relevance of these findings remains unclear. Recent findings indicate that Gaucher's disease and Parkinson's disease share pathophysiological features. Unique pathological findings, such as neuronal loss, astrogliosis, and the presence of intraneuronal Lewy-body–like synuclein inclusions specifically targeting the hippocampal CA2–3 region were recently described in both diseases.[29] Synuclein is a neuronal protein. Mutations in the gene encoding α-synuclein appear to be responsible for Parkinson's disease in rare familial cases, and the aggregated protein is a major component of Lewy bodies, the pathological hallmark of sporadic Parkinson's disease.[5] Thus, the presence of intraneuronal Lewy-body–like synuclein inclusions in patients with both type 1 and neuronopathic Gaucher's disease points to a selective vulnerability and cytotoxicity, specifically targeting the CA2–3 region that appears to characterize idiopathic Parkinson's disease, diffuse Lewy-body dementia, and according to recent reports, Gaucher's disease.

Carriage of type 1 Gaucher's disease is common in the Ashkenazi population. Taking into account the frequency of *GBA* mutations in the general Ashkenazi population and the general prevalence of parkinsonism,[30] we can extrapolate that the majority of carriers of mutant *GBA* alleles, in whom Parkinson's disease does not develop, are equipped with an efficient genetic mechanism that either prevents the deposition and accumulation of glucocerebroside in dopaminergic neurons or adequately degrades the glucocerebroside that is deposited. Alternatively, the occurrence of Parkinson's disease in carriers of Gaucher's disease may be accounted for by genetic variance in another gene.

In conclusion, our data indicate that some *GBA* mutations are genetic susceptibility factors for Parkinson's disease. We have also found that, in contrast to previous suggestions, heterozygosity for a non-neuropathic *GBA* mutation is not an absolutely asymptomatic state. Additional studies are needed to replicate our findings, to perform further analyses of the correlation between genotype and phenotype, and to identify the pathogenetic mechanisms that render some carriers of Gaucher's disease vulnerable to Parkinson's disease. The clinical implications of our findings and those of other studies that are soon to be completed should affect the treatment options available to patients with Parkinson's disease.

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