Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Genetics and Metabolism 81 (2004) 70–73
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Brief Communication

Glucocerebrosidase mutations in subjects with parkinsonism

Alicia Lwin, Eduard Orvisky, Ozlem Goker-Alpan, Mary E. LaMarca, and Ellen Sidransky*

Section on Molecular Neurogenetics, National Institute of Mental Health and Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda MD 20892-4405, USA

Received 13 November 2003; accepted 13 November 2003

Abstract

Recent studies showing an association between glucocerebrosidase deficiency and parkinsonism in Gaucher disease prompted an examination of the glucocerebrosidase gene sequence (GBA) and enzyme activity in brain samples from 57 subjects carrying the diagnosis of Parkinson disease. Alterations in GBA were identified in 12 samples (21%) and were more frequent among the younger subjects. These included eight with mutations (N370S, L444P, K198T, and R329C) and four with probable polymorphisms (T369M and E326K). Our findings suggest that mutations in glucocerebrosidase may be a risk factor for the development of parkinsonism.

Keywords: Gaucher disease; Parkinsonism; Glucocerebrosidase; Heterozygotes; Risk factors; Autopsy; Genotyping

Introduction

Gaucher disease (MIM 230800), the most common lysosomal storage disorder, results from the inherited deficiency of glucocerebrosidase (EC 3.2.1.45). There is growing evidence for an association between Gaucher disease and parkinsonism, demonstrated in recent studies of genotypically heterogeneous patients with relatively mild Gaucher disease who developed progressive parkinsonian manifestations, including tremor, bradykinesia and rigidity and often cognitive decline [1–5]. Neuropathologic studies revealed focal regions of gliosis, especially in the hippocampal C2–C4 region and calcarine cortex, and α-synuclein immunoreactive cortical and brainstem-type Lewy bodies [1,6]. These shared clinical and neuropathologic features prompted genotyping of the glucocerebrosidase gene (GBA) in autopsy samples from cases classified as Parkinson disease. Here we report that GBA mutations are encountered more often than expected in individuals with parkinsonism.

Materials and methods

Brain samples from subjects who carried a clinical or pathologic diagnosis of Parkinson disease were requested from five different brain bank repositories without additional prerequisites. Tissues from 57 probands were provided, with the age at death ranging from 54 to 99 years (mean age 76 years). One of the provided samples (Table 1, subject 2) also had the diagnosis of Gaucher disease. Tissues from 44 adult subjects without the diagnosis of Parkinson disease (mean age 70 years) were obtained from some of the same repositories as controls. DNA was extracted from frozen autopsy tissues and mutations in GBA were determined by sequencing the 11 exons and flanking intronic regions as previously described [1]. Brain extracts from probands and controls were screened for deficient enzyme activity using the standard 4-methylumbelliferyl-β-D-glucopyranoside assay [7].

Results and discussion

Alterations in GBA were identified in 12 of the 57 subjects with Parkinson disease (21%), including eight with known or novel mutations (two N370S

* Corresponding author. Fax: 1-301-402-6438.
E-mail address: sidranse@irp.nimh.nih.gov (E. Sidransky).

1096-7192/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2003.11.004

Table 1
Subjects with Parkinson disease and GBA alterations

Subject	Source	Age at diagnosis	Age of death	Pathologic findings	GBA genotype	Enzyme activity (% control)
1	MADRC	40s	54	LB[a] in SN, CC, HPC	N370S/N370S	7
2	MADRC	60s	75	LB[a] in SN, CC, HPC	N370S/N370S	11
3	MADRC	57	58	No LB	K198T/wt	43
4	MADRC	NA	68	LB in SN, CC	N370S/wt	50
5	PI	52	62	LB in SN, SCN	R329C/wt	64
6	HBTRC	63	75	LB in SN, CC-rare	N370S/wt	77
7	MADRC	56	73	LB in SN	N370S/wt	84
8	PI	48	70	LB in SN, CC, SCN, HA	L444P/wt	100
9	PI	60s	68	LB in SN, SCN, HA	T369M/wt	89
10	HBTRC	65	67	LB in SN, CC	T369M/wt	100
11	MADRC	81	86	LB[a]in SN, CC	T369M/wt	100
12	UMBTB	NA	72	No LB	E326K/wt	100

Abbreviations: *Tissue sources*: MADRC, Massachusetts Alzheimer Disease Research Center (total cases examined = 23); HBTRC, Harvard Brain Tissue Resource Center (15); PI, Parkinson Institute (10); UMBTB, University of Maryland Brain and Tissue Bank (6); CHTN, Cooperative Human Tissue Network (3-none positive). *Age*: NA, not available. *Pathology*: LB, Lewy body; SN, substantia nigra and/or locus ceruleus and dorsal motor nucleus of the vagus; CC, cerebral cortex. HPC, hippocampus; SCN, subcortical nuclei and/or nucleus basalis; HA, hypothalamus and amygdala. *Genotype*: wt, wildtype.

[a] LB, Lewy bodies were confirmed using antibodies to α-synuclein.

homozygotes, three N370S heterozygotes and one each carrying L444P, K198T, and R329C). The novel mutations K198T and R329C were confirmed on a second PCR product and/or by digestion with the enzyme *Aci*I. Four probands carried alterations that are presumed polymorphisms, T369M in three and E326K in one (Table 1). These changes are considered to be polymorphisms because they have been identified only in patients who carried another mutation on the same allele and have been found in 0.9–2% of control alleles [8,9]. Among the control subjects, two E326K carriers were identified, but no other mutations in GBA were found. Since the carrier frequency for Gaucher disease-causing alleles is estimated at 0.0343 in the high-risk Ashkenazi Jewish population and at approximately 0.006 in the general population [10], the frequency of GBA mutations identified in individuals with parkinsonism is higher than expected, and is greater than the reported incidence of mutations in other known Parkinson genes, including parkin and α-synuclein [11]. This, coupled with the clinical and neuropathological manifestations of parkinsonism observed both in patients with Gaucher disease and in their relatives [1,12], provides evidence that the two disorders are associated.

Among the 29 probands who died at age 75 or younger, eight (27%) were Gaucher heterozygotes or homozygotes (Fig. 1). At least five of the subjects with GBA mutations presented before age 60 and, in most, dementia and/or psychiatric symptoms developed. The pathology reports described cortical and/or hippocampal Lewy bodies in 62.5% of the subjects with GBA mutations, corresponding to the more aggressive parkinsonism described in probands with both Gaucher disease and parkinsonism [1].

We speculate that altered glucocerebrosidase, even in heterozygotes, may be a risk factor in the development of parkinsonism. This could be due to a "loss of function," where diminished enzymatic activity leads to a focal increase in glucosylceramide in specific brain regions. Excess glucosylceramide in cultured hippocampal neurons increases calcium release from intracellular stores, endoplasmic reticulum density, sensitivity to neurotoxic agents and activation of the ryanodine receptor, all of which can result in neuronal dysfunction [13]. Alternatively, since recent evidence suggests that α-synuclein is degraded by both autophagy and the proteosome [14], lysosomal dysfunction due to glucocerebrosidase deficiency might lead to improper degradation of α-synuclein and contribute to aggregate formation. Intraneuronal inclusions due to protein folding abnormalities are features common to Parkinson disease and other neurodegenerative disorders [15].

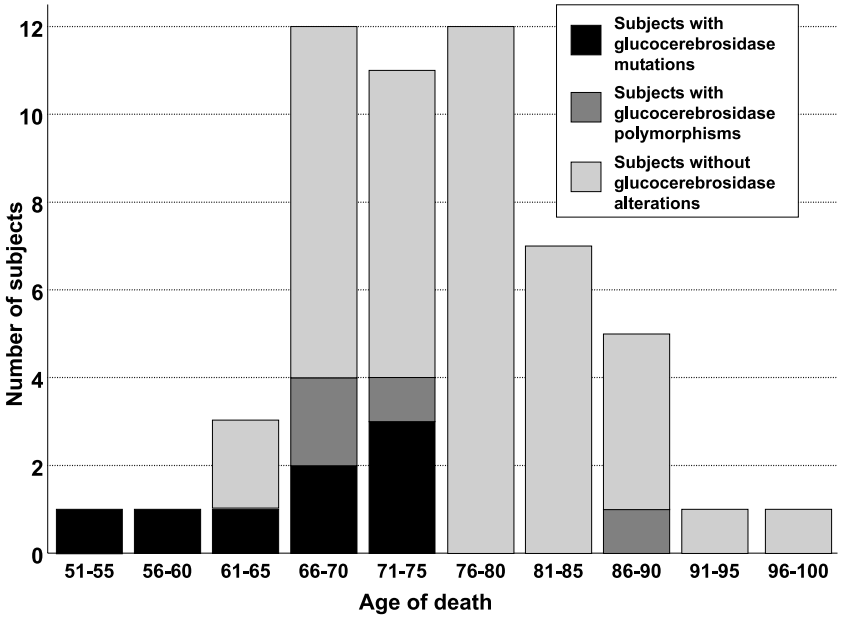

Fig. 1. Distribution of glucocerebrosidase alterations in Parkinson subjects by age of death.

Misfolding of aberrant glucocerebrosidase may affect lysosomal targeting and proteosome function, predisposing to synucleinopathies.

Nonetheless, the vast majority of patients with and carriers of Gaucher disease never develop clinical signs of parkinsonism, and even among those that do, there is not always concordance among sib pairs affected with Gaucher disease [1]. Clearly other modifiers, both genetic and environmental, must also be essential [16,17]. The mutated glucocerebrosidase may serve as a "second hit" in individuals who otherwise have an inherited predisposition to parkinsonism. Thus, the relative risk of developing parkinsonism in an individual with one or two GBA mutations is still unknown.

Here, studies of a rare metabolic disorder, Gaucher disease, may provide a window into both the genetics and pathogenesis of a common complex disorder, Parkinson disease. Further screening and better clinical assessment of subjects with parkinsonism, including panethnic cohorts with early onset, subjects of Ashkenazi Jewish extraction and relatives of patients with Gaucher disease, are necessary to better establish the full significance of this finding. Should the association persist, GBA mutations could be a significant inherited risk factor for the development of parkinsonism.

Acknowledgments

The technical assistance of Michael Eblan, Joann Nguyen, Shira Ziegler, and Barbara Stubblefield and secretarial help of Marie Hall are gratefully acknowledged. We also thank the Massachusetts Alzheimer Disease Research Center, the Harvard Brain Tissue Resource Center, the Cooperative Human Tissue Network, the University of Maryland Brain and Tissue Bank for Developmental Disorders and the Parkinson Institute for providing the tissues and pathology reports used in this study.

References

[1] N. Tayebi, J. Walker, B.K. Stubblefield, E. Orvisky, M.E. LaMarca, K. Wong, H. Rosenbaum, R. Schiffman, B. Bembi, E. Sidransky, Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism?, Mol. Genet. Metab. 79 (2003) 104–109.

[2] O. Neudorfer, N. Giladi, D. Elstein, A. Abrahamov, T. Turezkite, E. Aghai, A. Reches, B. Bembi, A. Zimran, Occurrence of Parkinson syndrome in type I Gaucher disease, Q. J. Med. 89 (1996) 691–694.

[3] M. Machaczka, M. Rucinska, A.B. Skotnicki, W. Jurczak, Parkinson syndrome preceding clinical manifestation of Gaucher disease, Am. J. Hematol. 61 (1999) 216–217.

[4] B. Bembi, S. Zambitu Marsalas, E. Sidransky, G. Ciana, M. Carrozzi, M. Zorzon, C. Martini, M. Gioulis, M. Pittis, L. Capus, Gaucher disease with Parkinson disease: clinical and pathologic aspects, Neurology 61 (2003) 99–101.

[5] J. Várkonyi, H. Rosenbaum, N. Baumann, J.J. MacKenzie, Z. Simon, J. Aharon-Peretz, J.M. Walker, N. Tayebi, E. Sidransky, Gaucher disease associated with parkinsonism: four further case reports, Am. J. Med. Genet. 116A (2003) 348–351.

[6] K. Wong, E. Sidransky, T. Mixon, L.K. Wakefield, G.D. Sandberg, A. Morrison, C. Colegial, B. Kaya, A.H. Futerman, R. Schiffmann, Neuropathology of calcarine cortex layer 4B and hippocampal CA2-4 regions with brain stem-type Lewy bodies and sparing of CA1: a study of 14 patients with Gaucher disease, J. Neuropathol. Exp. Neurol. 62 (2003) 12.

[7] S.P. Peters, P. Coyle, R.H. Glew, Differentiation of beta-glucocerebrosidase from beta-glucosidase in human tissues using sodium taurocholate, Arch. Biochem. Biophys. 175 (1976) 569–582.

[8] J.K. Park, N. Tayebi, B.K. Stubblefield, M.E. LaMarca, J.J. MacKenzie, D.L. Stone, E. Sidransky, The E326K mutation and Gaucher disease: mutation or polymorphism?, Clin. Genet. 61 (2003) 32–34.
[9] J.M. Walker, A. Lwin, N. Tayebi, M.E. LaMarca, E. Orvisky, E. Sidransky, Glucocerebrosidase mutation T369M appears to be another polymorphism, Clin. Genet. 63 (2003) 237–238.
[10] E. Beutler, G.A. Grabowski, Gaucher disease, in: C.R. Scriver, A.L. Beaudet, D. Valle, W.S. Sly (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, pp. 3635–3668.
[11] R.L. Nussbaum, C.E. Ellis, Alzheimer's disease and Parkinson disease, N. Engl. J. Med. 348 (2003) 1356–1364.
[12] O. Goker-Alpan, A. Lwin, E. Sidransky, Do mutations in glucocerebrosidase modify the course of Parkinson disease? Family studies in patients with Gaucher disease, Am. J. Hum. Genet. 73 (2003) 449.
[13] E. Korkotian, A. Schwarz, D. Pelled, G. Schwarzmann, M. Segal, A.H. Futerman, Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons, J. Biol. Chem. 274 (1999) 21673–21678.
[14] J.L. Webb, B. Ravikumar, J. Atkins, J.N. Skepper, D.C. Rubinsztein, α-Synuclein is degraded by both autophagy and the proteasome, J. Biol. Chem. 278 (2003) 25009–25013.
[15] J.Q. Trojanowski, M. Goedert, T. Iwatsubo, V.M. Lee, Fatal attractions: abnormal protein aggregation and neuron death in Parkinson disease and Lewy body dementia, Cell Death Differ. 5 (1998) 832–837.
[16] C.R. Scriver, P.J. Waters, Monogenic traits are not simple: lessons from phenylketonuria, Trends Genet. 15 (1999) 267–272.
[17] K.M. Dipple, E.R. McCabe, Phenotypes of patients with simple mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics, Am. J. Hum. Genet. 66 (2003) 1729–1735.