50% of PD patients develop dementia (PDD) after no less than 10 to 15 years following PD diagnosis [5].

Glucocerebrosidase (GCase) is a lysosomal enzyme responsible for the breakdown of glucocerebroside into glucose and ceramide [6]. Mutations in the GCase gene *GBA* cause GCase deficiency leading to glucocerebroside accumulation inside the lysosome. This accumulation results in Gaucher disease, the most frequent lysosomal storage disorder [6]. Since several probands with Gaucher disease present parkinsonism [7] and have *GBA* mutation-carrier relatives with PD [8], subsequent studies have revealed that *GBA* mutations are strongly associated with PD but also with DLB [9-11].

Analyses of GCase activity and expression levels in PD brains have shown that GCase activity and protein levels are diminished in sporadic PD with and without *GBA* mutations [12, 13]. Furthermore, decreased GCase activity has been found in blood of PD patients [14]. Decrease of GCase activity causes the accumulation of glucocerebroside in lysosomes, directly promoting AS oligomerization and fibrillation. At the same time, AS fibrils inhibit GCase activity creating a bidirectional pathogenic loop [15].

Over the past years, deregulation of alternative splicing has been described repeatedly as an important mechanism involved in ageing and disease development [16, 17]. In this context, we have reported that differential isoform expression changes are involved in LBD pathogenesis [18, 19]. For the GBA gene, five transcript variants (tv; http://www.ncbi.nlm.nih.gov/gene/2629) have been reported by the NCBI database. GBAtv1-3 are the result of alternative inclusion of their initial exons encoding the same protein. GBAtv4 and tv5 are the result of splicing out of exons 2 and 3 or exon 5, and bear shorter proteins.

In this study, we addressed three main questions. First, we wanted to know if GCase deficiency in LBD starts at the transcriptional level; second, if possible brain GBA expression changes are also detectable in blood of LBD patients and third, if alternative *GBA* splicing is dysregulated in these patients.

MATERIALS AND METHODS

Brain tissues

Post-mortem brain samples and their corresponding clinical and neuropathological diagnoses were provided by the Institute of Neuropathology Brain Bank and the Neurological Tissue Bank of the University of Barcelona / Hospital Clinic, Barcelona, Spain. They were obtained from 20 patients with clinical diagnosis of DLB, 25 patients with clinical diagnosis of PD, and 17 donors devoid of neurological signs or symptoms and lack of neuropathological findings. Eight of the DLB brains did not present AD-related pathology and were defined as pure DLB (pDLB), while 12 DLB brains contained concomitant AD-related pathology and were considered as common DLB (cDLB). Of the 25 PD patients, 13 developed dementia (Parkinson's disease with dementia; PDD) but 12 did not (Parkinson's disease without dementia; PDND). None of the patients included in this study carried GBA mutations. Neuropathological diagnosis was carried out as described before [20]. Two brain areas, temporal cortex and caudate nucleus, were analyzed for all disease groups, and the pons was available for PD only. Frontal cortex samples were used to estimate relative expression levels of GBA transcripts. Clinical and neuropathological characteristics of patients and controls are summarized in Table 1.

Table 1. Clinico-neuropathological characteristics of Lewy body disease cases and controls.

Disease	n	PMtime[1] (range)	ADstage[2]	Br&Br[3]	Death[4] (range)	M:F ratio[5]
pDLB[6]	8	9:30 (3:30-17:00)	0-II	A-C	74.6 (60-85)	3:1
cDLB[7]	12	10:30 (4:00-21:15)	III-VI	B-C	79.0 (74-86)	1.4:1
PD[8]	12	7:00 (3:30-14:00)	III-IV		80.8 (68-93)	1:1
PDD[9]	13	7:10 (4:00-12:20)	II-VI	A-C	78.7 (71-87)	0.9:1
CTRL[10]	17	8:40 (2:30-23:30)			69.3 (55-81)	1.4:1

[1] post-mortem time; [2] AD stages following Braak and Braak, I-VI: neurofibrillary tangles; [3] AD stages following Braak and Braak, A-C: amyloid plaques; [4] death, age at death; [5] M:F ratio, male-female ratio; [6] pDLB, dementia with Lewy bodies, pure form; [7] cDLB, common dementia with Lewy bodies; [8] PD, Parkinson disease without dementia; [9] PDD, Parkinson disease with dementia; [10] CTRL, control brain samples.