Glucocerebrosidase inhibition causes mitochondrial dysfunction and free radical damage

Michael W.J. Cleeter, Kai-Yin Chau, Caroline Gluck, Atul Mehta, Derralynn A. Hughes, Michael Duchen, Nicholas William Wood, John Hardy, J. Mark Cooper, Anthony Henry Schapira

Department of Clinical Neurosciences, University College London Institute of Neurology, United Kingdom
Departments of Cell and Developmental Biology, United Kingdom

Corresponding author. Address: Department of Clinical Neurosciences, UCL University College London, Department of Cell and Developmental Biology, United Kingdom

ABSTRACT

Mutations of the gene for glucocerebrosidase 1 (GBA) cause Gaucher disease (GD), an autosomal recessive lysosomal storage disorder. Individuals with homozygous or heterozygous (carrier) mutations of GBA have a significantly increased risk for the development of Parkinson’s disease (PD), with clinical and pathological features that mirror the sporadic disease. The mechanisms whereby GBA mutations induce dopaminergic cell death and Lewy body formation are unknown. There is evidence of mitochondrial dysfunction and oxidative stress in PD and so we have investigated the impact of glucocerebrosidase (GCase) inhibition on these parameters to determine if there may be a relationship of GBA loss-of-function mutations to the known pathogenetic pathways in PD. We have used exposure to a specific inhibitor (conduritol-b-epoxide, CbE) of GCase activity in a human dopaminergic cell line to identify the biochemical abnormalities that follow GCase inhibition. We show that GCase inhibition leads to decreased ADP phosphorylation, reduced mitochondrial membrane potential and increased free radical formation and damage, together with accumulation of alpha-synuclein. Taken together, inhibition of GCase by CbE induces abnormalities in mitochondrial function and oxidative stress in our cell culture model. We suggest that GBA mutations and reduced GCase activity may increase the risk for PD by inducing these same abnormalities in PD brain.

1. Introduction

Glucocerebrosidase 1 (GCase) is a ubiquitous lysosomal enzyme responsible for the breakdown of glucocerebroside to glucose and ceramide. Diverse mutations within the gene (GBA) that encodes GCase result in mutant enzymes with reduced activity and an autosomal recessive storage disorder (Gaucher disease – GD). GD patients have reduced GCase activity while heterozygote carriers generally have an intermediate level (Raghavan et al., 1980). GD is characterised by widespread accumulation of the GCase substrates glucocerebroside or glucosylsphingosine in many organs (Grabowski, 2008). Although GBA mutations cause a reduction in enzyme activity, this may not necessarily be the mechanism that mediates the pathogenesis of GD and alternative models include mis-trafficking of GCase and endoplasmic reticulum stress (Kov-Bar et al., 2011).

Alpha-synuclein positive Lewy bodies have been identified in the brains of GD patients and carriers who died with PD (Neumann et al., 2009; Wong et al., 2004). There are now persuasive data that GBA mutations are a major risk factor for PD and result in a clinical and pathological phenotype that is virtually indistinguishable from sporadic PD (Sidransky et al., 2009). The mechanism(s) whereby GBA mutations increase the risk for PD remain unidentified. PD pathogenesis is thought to involve a number of pathways including mitochondrial dysfunction and oxidative stress (Schapira, 2006). Given the similar clinical and pathological phenotypes of GBA–PD and sporadic PD, we hypothesised that reduced GCase activity would result in biochemical events that would map to these same pathways considered of pathological relevance to familial and sporadic PD. We therefore investigated the effects of a specific GCase inhibitor (conduritol-b-epoxide) on mitochondrial function and free radical generation.

2. Materials and methods

2.1. Reagents

Reagents were supplied by Sigma–Aldrich (Poole, UK) and Merck (Nottingham, UK) unless otherwise stated.

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* Corresponding author. Address: Department of Clinical Neurosciences, UCL Institute of Neurology, Rowland Hill St., London NW3 2PF, United Kingdom. Tel.: +44 020 7830 2012; fax: +44 020 7472 6829. E-mail address: a.schapira@ucl.ac.uk (A.H. Schapira).
1 These authors contributed equally to the work.

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2.2. Cell cultures and treatments

SHSY-5Y cells were maintained as described (Alvarez-Erviti et al., 2010). During the course of continuous conduritol-β-epox ide (CβE; Universal Biologicals, Cambridge, UK) treatment, cells were split 1:3 every 3 days with fresh CβE added to a final concentration of 50 μM. GCCase activity was monitored at different times throughout the course of the experiment to check that inhibition was maintained. Cell viability was checked by lactate dehydrogenase (LDH) release assays (Roche, UK). In brief, cells were plated into pairs of wells of a 48-well dish in phenol red-free media. After 48 h incubation of the control and CβE treatment, Triton X-100 was added to the medium of one of each pair of wells to a final concentration of 1% and the LDH level in the medium served as its total level. The LDH level found in the medium of the other well represents released LDH. Results were expressed as percentage LDH release from the total. For other assays, cells were harvested by trypsinisation. If required for assay of GCCase, mitochondrial respiratory chain (MRC), or aconitate activities, cells were centrifuged at 1000 rpm, washed twice with phosphate-buffered saline (PBS) and stored as a pellet at −80 °C until assayed. Cells were then resuspended in 0.25 mM sucrose, 50 mM Tris (hydroxymethyl) amino methane hydrochloride (Tris–HCl) pH 7.4 and freeze–thawed three times prior to assay. If required for adenosine diphosphate (ADP) phosphorylation measurements, cell numbers were determined using haemocytometers (Immune Systems; Paisington, UK), and cells were centrifuged and resuspended in buffer for assays (see below).

2.3. Enzyme assays and ATP synthesis measurements

Respiratory chain (Schapira et al., 1990), proteasome (Schapira et al., 2006), aconitase (Bradley et al., 2000) and ADP phosphorylation activities (Korlpira et al., 2004) were measured by standard techniques as described previously. CβE-sensitive GCCase activity (end-point measurement) was determined at 37 °C essentially as described (Preence et al., 1996) using 4-Methylumbelliferyl-β-D-glucopyranoside as substrate in a plate reader (‘Synergy’, LabTech; Brighton, UK). The increase in fluorescence of released 4-Methyl umbelliferalone at 460 nm following excitation at 360 nm was followed over 1 h. Protein levels were estimated using a bichoninic acid (BCA) kit (Pierce Thermo Fisher; Paisington, UK) with reference to the protein standard supplied with the kit.

2.4. Western blot analysis

Cells were harvested, washed with PBS and processed as described (Alvarez-Erviti et al., 2010). 25–40 μg of whole cell lysates were electrophoresed on Novex gels (NuPage 4–12%; Invitrogen, Paisley, UK) and transferred onto polyvinylidene fluoride membrane (Millipore; Watford, UK) and then probed with antibodies to porin (Merck; Darmstadt, Germany; 1/25000 dilution), adenine nucleotide transporter (ANT; Abcam, UK, 1/1000 dilution), microtubule-associated protein 1 light chain 3 (LC3, Clone D11; Cell Signalling, USA, 1/1000 dilution), alpha-synuclein (Becton–Dickinson, UK, 1/500 dilution), Glucocerebrosidase (GBA; Abcam, UK, 1.1000 dilution) or lysosome-associated membrane proteins (lamp1, clone H4A3; Abcam, UK, 1/1000 dilution) and all were normalised to β-actin (Abcam; 1/5000 dilution) blots were developed using an enhanced chemiluminescence (ECL) kit (GE Healthcare; Little Chalfont, UK), exposed to X-ray film (GE Healthcare). The film was developed and signal intensities in the linear range were quantified by the ‘Alphagel’ software package (Alphalnnotech; San Landro, USA).

2.5. Live cell confocal imaging and analysis

Fluorescence of cells grown on 22 mm coverslips were measured by real-time confocal imaging as described (Gandhi et al., 2009; Duchen et al., 2003), using a Zeiss 510 laser scanning microscope equipped with an additional Enterprise UV laser source and a cooled charge coupled device camera, bathed in standard phenol red-free Hank’s Buffered Salt Solution at room temperature. Mitochondrial membrane potential (Ψm) was quantified by steady-state fluorescence (excited using the 543 nm laser line and measured using a 560 nm longpass filter) of mitochondrial patterns produced by 25 nM tetramethyl rhodamine methyl ester (TMRE; Invitrogen) stained at room temperature for 45 min. Free radical generation was measured by the rate of ratiometric change of reduced and oxidised dihydroethidium (DHE; Invitrogen) fluorescence. 10 μM of DHE was loaded at room temperature, and measurements were made typically over 120 s. Oxidised DHE was measured with the 543 nm laser line and 560 nm long-pass filter, while for reduced DHE measurement it was excited at 351 and measured at 435–485 nm. Cells were treated accordingly and measured at the same time to minimise variability of fluorescence measurements. Individual cells were marked and mean fluorescence of individual cells measured by the ImageJ software (NIH, USA); where the image was captured with Z-stack, z-projection was performed using max intensity and net fluorescence was obtained by subtracting the background fluorescence. Mitochondrial morphology was measured on the circularity and aspect ratio from the TMRE images by ImageJ as described (Wang et al., 2011).

2.6. Creation of GBA knockdown SHSY-5Y stable cell lines

SHSY-5Y cells were transfected with a ‘Hush’ GBA knockdown plasmid (Origene, USA), empty plasmid and scrambled control (The sequence chosen for the GBA knockdown was: GTGTCGTGTGCAATGCCCACATGCTGTA). Stable clones were isolated following selection with puromycin (Sigma, UK) at 4 μg/ml and characterised by analysis of GCCase activity, actin-normalised GBA mRNA by a ‘StepOne’ QPCR machine (Applied Biosystems, UK) using SyBr Green (Life Technologies, UK) and appropriate primers for GBA and β-actin (Eurofins, Germany) and GCase protein levels (by Western blotting). Clones were assessed after several passages (in the presence of a maintenance dose of 2 μg/ml puromycin) to check for the continuation of any knockdown effect.

2.7. Statistical analysis

Where multiple comparisons were made, one-way ANOVA tests were performed followed by Dunnett post test analysis in order to determine statistical significance. Student’s t-tests were used for comparing statistical significance between 2 populations. A p value of < 0.05 was considered as statistically significant.

3. Results

3.1. CβE

CβE has been reported to be a selective inhibitor of GCCase activity (Preence et al., 1996; Newburg et al., 1986) and we have confirmed in SHSY-5Y cells that 50 μM CβE decreased GCCase activity to ≤5% of untreated cells and maintained the inhibition of GCCase activity over 30 days (Suppl. Fig. 1). This concentration of CβE has also been previously reported to result in a greater than 2-fold increase of glucocerebrosidase over 24 days (Preence et al., 1996). In our experiments, 30 days CβE treatment had no effect on cell viability as judged by LDH release (Suppl. Fig. 2).
3.2. Mitochondrial studies

3.2.1. ATP synthesis (ADP phosphorylation)

Fig. 1 shows the ADP phosphorylation capacity of digitonin-permeabilised cells following incubation with CβE. There was no measurable effect before 10 days, but complex I-linked ADP phosphorylation with glutamate/malate as substrate was significantly decreased by 47% at 20 days (p < 0.01) and by 33% at 30 days (p < 0.05), compared to control. Complex II/III-linked ADP phosphorylation using succinate as substrate was significantly reduced by 30% at 20 days (p < 0.05) and 26% at 30 days (p < 0.05). There was no significant change in complex IV-linked ADP phosphorylation with ascorbate/TMPD as substrate. Basal levels of ATP (determined from time zero acid-treated samples) were 616 ± 149 and 643 ± 270 pmol/million cells, for control and 30 day treated cells respectively. These values represented approximately 1.5–4.5% of the values determined for ADP phosphorylation with glutamate/malate over the incubation period.

3.2.2. Respiratory chain activities

Table 1

| Assay                | Control | 30 days CβE |
|----------------------|---------|-------------|
| (a) MRC activities   |         |             |
| CXII/CIII × 100      | 13.4 ± 0.6 | 14.6 ± 3.1  |
| CXIV/CIII × 1000     | 4.3 ± 1.1  | 4.6 ± 0.8   |
| (b) Proteasome activities |
| PCP-like             | 1.6 ± 0.6  | 2.5 ± 0.25  |
| Chymotrypsin-like    | 6.0 ± 1.8  | 5.7 ± 2.0   |
| Trypsin-like         | 8.7 ± 2.9  | 8.6 ± 1.4   |

* mFluorescence units/min/mg protein × 10⁻⁶.

20% at 10 days (p < 0.01), 23% at 20 days (p < 0.01) and 28% at 30 days (p < 0.01). We also observed fragmentation of the mitochondrial network at 30 days (Fig. 2B–D).

3.2.4. Mitochondrial content

Western blot analysis of the mitochondrial marker proteins porin and adenine nucleotide transporter (ANT), normalised to actin as a loading control, showed no significant change with CβE. This indicates that CβE treatment for up to 30 days had no significant effect on mitochondrial content (Fig. 2E and F).

3.3. Oxidative stress

DHE fluorescence was used to measure reactive oxygen species generation in live cells, for example as demonstrated for the free radical generator paraquat (Fig. 3A). CβE caused a significant increase in the rate of DHE oxidation by 52% at 20 days (p < 0.01) and by 71% at 30 days (p < 0.01). Aconitase activity, a measure of free radical mediated damage, was reduced by 60% (p < 0.001) after 30 days incubation with CβE (Fig. 3B).

3.4. Protein degradation studies

3.4.1. (A) Ubiquitin Proteasomal System (UPS)

The accumulation of alpha synuclein and other proteins in Lewy bodies is a characteristic feature of PD pathology. The UPS has been reported to be abnormal in PD brain and therefore potentially to contribute to protein aggregation (McNaught and Jenner, 2001). We investigated the effect of GCase inhibition on proteasomal function, but found no abnormalities (Table 1). We did observe a significant increase (59%, p < 0.002) in alpha synuclein levels in the treated cells, as determined by Western blotting (Fig. 4A and B). Using alpha-synuclein fused to green fluorescent protein (GFP), at 20 days there was a 49% increase in levels as reflected by fluorescence in SH-SY5Y cells following CβE (Suppl. Fig. 3).

3.4.2. (B) Lysosomal studies

An indication that the lysosomal content of the cells was unchanged is shown in Fig. 5A and B: Lamp1 levels (normalised to actin) showed no significant difference between control cells and CβE treated ones. Western blots of LC3-II on SH-SY5Y cells did not show a change in the levels of LC3-II when GCase was inhibited by CβE (Fig. 6A and B), Bafilomycin treatment (100 nM for 3 h) of these cells did not appear to increase the observed levels significantly.

3.5. GBA knockdown

To confirm the effects of GCase inhibition by CβE, we generated a stable shRNA-mediated knockdown model of GBA in SH-SYSY cells. Suppl. Fig. 4A shows that the enzyme activity was reduced by 62% and Western blot band densities indicated that the level of protein...
was decreased by 59% (Suppl. Fig. 4B and C), compared to the scrambled control levels. Quantitative PCR data also showed a significant decrease of 60% in the mRNA for GBA relative to the scrambled control (data not shown). As shown in Suppl. Fig. 4D, knockdown of GBA caused a significant fall in TMRM fluorescence ($p < 0.01$ after ANOVA followed by Dunnett post test analysis), (B) Representative TMRM fluorescence images of untreated and SHSY-5Y cells treated with CβE for 30 days showing disruption of the mitochondrial network. (C) Image analysis reveals that both the circularity and (D) aspect ratio of the mitochondria upon CβE treatment are significantly increased ($p < 0.0001$ by t-test), suggesting the mitochondria are more fragmented (solid bars: control; open bars: treated cells. (E) Levels of porin and ANT were measured in SHSY-5Y cells incubated with 50 µM CβE for 30 days (lanes 4–6) by Western blot analysis, against untreated cells (lanes 1–3) Blot (i) upper panel: β-actin; lower panel: ANT. Blot (ii) upper panel: β-actin; lower panel: porin. Graph (F) shows that there was no significant effect on porin or ANT levels (mean ± SEM, n = 3) by this treatment, (black bars, control; open bars: treated cells).

3.6. Other models of lysosomal inhibition

Inhibition of lysosomal function by bafilomycin or ammonium chloride produced a different pattern of impaired ADP
phosphorylation (bafilomycin) or no effect (ammonium chloride) (Suppl. Fig. 7A). These inhibitors also showed different effects to CβE on mitochondrial membrane potential (Suppl. Fig. 7B). These results support our data that the mitochondrial effects of CβE are not mediated directly through lysosomal inhibition.

Fig. 3. CβE treatment led to increased free radical production and reduced aconitase activity. (A) As a positive control, paraquat pre-treatment at 300 μM for 1 day (cross-hatched bar; ‘+PQ’) significantly increased the rate of DHE oxidation, as analysed by paired t-tests (p = 0.002). Continuous treatment of SHSY-5Y cells with 50 μM CβE (open bars) showed a progressive increase in the rate of DHE oxidation after 20 days (⁄p < 0.01, mean percentage changes with respect to the untreated control (solid bar) ±SEM) as determined by one-way ANOVA followed by Dunnett post test. (B) Aconitase activity was significantly reduced (to 37% of control) after 30 days of CβE treatment (mean ± SEM, “p < 0.001).

Fig. 4. CβE treatment led to increased alpha-synuclein. Levels of alpha-synuclein were measured in SHSY-5Y cells incubated with 50 μM CβE for 30 days by Western blot analysis, against untreated cells. (A) Shows the blots of untreated (lanes 1–3) or CβE-treated (lanes 4–6) SHSY-5Y cells, stained with anti-alpha-synuclein (lower panels) or anti-β-actin (upper panels). (B) Shows the graphical representation of alpha-synuclein levels from the scanned blots, normalised to β-actin levels (solid bar: control, open bar: treated cells). The level of alpha-synuclein protein was significantly (⁄p < 0.002; n = 3) higher (by 59%) than that of control levels.

Fig. 5. CβE treatment did not affect Lamp 1 levels. Levels of Lamp 1 were estimated by Western blotting of control cells (lanes 1–3) and treated ones (lanes 4–6) with anti-Lamp 1 and normalising with anti-β-actin. (A) Shows the blot and (B) shows a graphical representation of the result (solid bar: control, open bar: treated). No significant difference was seen between the two groups (n = 3).
4. Discussion

The aetiopathogenesis of PD is thought to involve an interaction of genetic and environmental factors and includes mitochondrial dysfunction, oxidative stress and protein handling abnormalities, as well as inflammation (Schapira and Tolosa, 2010; Schapira, 2012). Specific gene mutations have been identified as causes of familial PD and genome-wide association studies have highlighted the importance of alpha-synuclein and tau as contributors to PD risk. The genetic causes of familial PD are thought to initiate biochemical events that map to these same pathways. GBA mutations have now been reproducibly associated with a substantially increased risk for PD estimated variously as 5 to 20-fold (Sidransky et al., 2009; Bultron et al., 2010).

We have followed over time the effects of GCase enzyme inhibition and knockdown on mitochondrial function and oxidative stress. In our cell model, the first change in function we observed following CβE exposure was a progressive decline in mitochondrial membrane potential that reached significance at 10 days. Our studies then showed that inhibition of GCase activity to a degree comparable to that seen in GD (Raghavan et al., 1980), caused a significant reduction of ATP synthesis involving both complex I-linked and complex II/III-linked ADP phosphorylation although the individual respiratory chain enzyme activities can be seen in certain mitochondrial diseases and suggests a defect of electron transfer or mitochondrial membrane defect (Schapira, 2012). Although CβE is considered a specific inhibitor of GCase, we cannot completely exclude the possibility of off-target effects or secondary pathways initiated by GCase inhibition inducing the effects seen. However, GBA knockdown leading to a 62% reduction in enzyme activity induced a similar pattern of changes with a decrease in mitochondrial membrane potential and a significant fall in aconitase activity. Furthermore, the increase in oxidative stress observed here is compatible with a previous observation from GD fibroblast lines of an increase in superoxide ions generated from non-phagocytic NADPH oxidase and an associated increase in protein oxidation in the form of protein carbonyls (Degnuto et al., 2007).

Recent data have supported a role for defective lysosomal-dependent degradation (autophagy) in PD. Abnormalities of the chaperone-mediated autophagy (CMA) pathway have been demonstrated in PD brain in anatomic regions that map to neuronal degeneration and Lewy body deposition (Alvarez-Erviti et al., 2010). GBA mutations would be expected to reduce GCase activity in the lysosome and lead to the accumulation of glucocerebroside and glucosylsphingosine. A direct interaction between GCase and alpha-synuclein under lysosomal conditions has recently been described (Yap et al., 2011). It is notable that GBA–PD pathology is alpha-synuclein Lewy body-positive, and alpha-synuclein is predominantly metabolised by CMA. The accumulation of alpha-synuclein in GBA-associated PD may therefore reflect reduced turnover secondary to impaired CMA or lysosomal function. Knockdown of GCase has been shown to reduce the rate of proteolysis in cells by 40% through disruption of the lysosomal pathway (Mazzulli et al., 2011). Alpha synuclein steady state levels were increased in GD cells and GCase knockout models with an accumulation of a soluble high molecular weight form of the protein. Alpha synuclein aggregates have also been seen in a mouse model of GD (Sardi et al., 2011). It is of interest therefore that CβE inhibition of GCase activity in our cell model caused an increase in alpha-synuclein levels. This has also been reported following CβE treatment in mouse substantia nigra (Manning-Bog et al., 2009). We have recently studied post-mortem brain samples from PD patients with and without GBA mutations (Gegg et al. in press). In addition to reduced GCase activity in both GBA positive and negative brains,
Most profound in substantia nigra, we demonstrated in vivo and in vitro features supportive of the reciprocal relationship between alpha-synuclein and GCase activity. Thus, the lower the activity of GCase, the higher the alpha-synuclein levels, and the greater the expression of alpha-synuclein the lower the GCase activity.

The results from the present study indicate that inhibition of GCase induces defects in mitochondrial function, increases oxidative stress and confirms previous reports that inhibition of this enzyme increases alpha-synuclein levels. These abnormalities are also found in the PD brain, particularly in the substantia nigra, the main site of neuronal degeneration. We suggest that GBA mutations and decreased GCase activity increase the risk for PD by inducing or exacerbating these same abnormalities of mitochondrial function, oxidative stress and alpha-synuclein accumulation to cause or accelerate the development of PD.

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Appendix A. Supplementary data

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References

Alvarez-Ervti, I., Rodriguez-Oroz, M.C., Cooper, J.M., Caballero, C., Ferrer, I., Obeso, J.A., Schapira, A.H., 2010. Chaperone-mediated autophagy markers in Parkinson disease brains. Arch. Neurol. 67, 1464–1472.
Bradley, J.L., Blake, J.C., Chamberlain, S., Thomas, P.K., Cooper, J.M., Schapira, A.H., 2000. Clinical, biochemical and molecular genetic correlations in Friedreich's ataxia. Hum. Mol. Genet. 9, 275–282.
Bultron, G., Kacena, K., Pearson, D., Boxer, M., Yang, R., Sathe, S., Pastores, G., Mistry, P.K., 2010. The risk of Parkinson's disease in type 1 Gaucher disease. J. Inherit. Metab. Dis. 33, 167–173.
Degano, M., Pittis, M.G., Pines, A., Dominissini, S., Kelley, M.R., Garcia, R., Quadrifoglio, F., Bembi, B., Tell, G., 2007. Altered intracellular redox status in Gaucher disease fibroblasts and impairment of adaptive response against oxidative stress. J. Cell. Physiol. 212, 223–235.
Duchen, M.R., Surin, A., Jacobson, J., 2003. Imaging mitochondrial function in intact cells. Methods Enzymol. 361, 353–389.
Gandhi, S., Wood-Kaczmar, A., Vao, Z., Plun-Favreau, H., Deas, E., Klupsch, K., Downward, J., Latchman, D.S., Tabrizi, S.J., Wood, N.W., Duchen, M.R., Abramov, A.Y., 2009. Pink1-associated Parkinson’s disease is caused by neuronal vulnerability to calcium-induced cell death. Mol. Cell 33, 627–638.
Gegg, M.E., Vurke, D., Heales, S.J.R., Cooper, J.M., Hardy, J., Wood, N.W., Schapira, A.H.V., in press. Glucocerebrosidase deficiency in substantia nigra of Parkinson’s disease. Neuror. Lett. 297, 191–194.
Neumann, J., Bras, J., Deas, E., O’Sullivan, S.S., Parkinlins, L., Lachmann, R.H., Li, A., Holton, J., Guerreiro, R., Fauld, P., Segarane, R., Singleton, A., Lees, A., Hardy, J., Houlden, H., Revesz, T., Wood, N.W., 2009. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson’s disease. Brain 132, 1783–1794.
Newburg, D.S., Yatiziv, S., McCluer, R.H., Raghavan, S., 1986. Beta-Glucocerebrosidase inhibition in murine peritoneal macrophages by conduritol-b-epoxide: an in vitro model of the Gaucher cell. Biochim. Biophys. Acta 877, 121–126.
Precon, E.M., Chaturvedi, P., Newburg, D.S., 1996. In vitro accumulation of glucocerebrosidase in neuroblastoma cells: a model for study of Gaucher disease pathobiology. J. Neurosci. Res. 43, 365–371.
Raghavan, S.S., Topol, J., Solodky, E.H., 1980. Lysosome beta-glucosidase in homoyzygotes and heterozygotes for Gaucher disease. Am. J. Hum. Genet. 32, 158–173.
Sardi, S.P., Clarke, J., Kimnecom, C., Tamsett, T.J., Li, L., Stanek, L.M., Passini, M.A., Grabowski, G.A., Schlossmacher, M.G., Sidd, R.M., Cheng, S.H., Shibabuddin, L.S., 2011. CNS expression of glucocerebrosidase corrects alpha-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. Proc. Natl. Acad. Sci. USA 108, 12101–12106.
Schapira, A.H., 2006. Etiology of Parkinson’s Disease. Neurology 66, S10–S23.
Schapira, A.H., 2012. Mitochondrial diseases. Lancet 379, 1825–1834.
Schapira, A.H., Tolosa, E., 2010. Molecular and cellular prorome of Parkinson disease: implications for treatment. Nat. Rev. Neurol. 6, 309–317.
Schapira, A.H., Mann, V.M., Cooper, J.M., Dexter, D., Daniel, S.E., Jenner, P., Clark, J.B., Marsden, C.D., 1990. Anatomic and disease specificity of NADH COQ1 reductase (complex I) deficiency in Parkinson’s disease. J. Neurochem. 55, 2142–2145.
Schapira, A.H., Cleeter, M.W., Muddle, J.R., Workman, J.M., Cooper, J.M., King, R.H., 2006. Proteasomal inhibition causes loss of nigral tyrosine hydroxylase neurons. Ann. Neurol. 60, 253–255.
Sidransky, E., Nalls, M.A., Aylott, J.O., Haron-Perez, J., Annesi, G., Barbossa, E.R., Bar-Shira, A., Berg, D., Bras, J., Price, A., Ch, C.M., Clark, L.N., Condroyer, C., De Marco, E.V., Duri, A., Elhan, M.J., Fahn, S., Farrer, J., Fung, H.C., Gan-Or, Z., Gasser, T., Gershoni-Baruch, R., Giladi, N., Griffith, A., Gurevich, T., Januario, C., Kropp, P., Lang, A.E., Lee-Chen, G.J., Lesage, S., Marder, K., Muta, I.F., Mirelman, A., Morz, J., Mzuta, I., Nicoletti, G., Oliveira, C., Ottman, R., Ott-Unterreg, A., Pereira, L.V., Quattrone, A., Rogaeva, E., Rolf, A., Rosenbaum, H., Rozenberg, R., Sami, A., Samaddar, T., Schulte, C, Sharma, M., Singleton, A., Spitz, M., Tan, E.K., Tayebi, N., Toda, T., Troiano, A.R., Tsuji, S., Wittstock, M., Wolfsberg, T.G., Wu, Y.R., Zabetian, C.P., Zhao, Y., Ziegler, S.G., 2009. Multicenter analysis of 2011. DLP1-dependent mitochondrial fragmentation mediates 1-methyl-4-phenylpyridinium toxicity in neurons: implications for Parkinson disease. Aging Cell. 11, 807–823.
Wang, X., Su, B., Liu, W., He, X., Gao, Y., Castellani, C.J., Perry, G., Smith, M.A., Zha, X., 2011. DLP1-dependent mitochondrial fragmentation mediates 1-methyl-4-phenylpyridinium toxicity in neurons: implications for Parkinson's disease. Aging Cell. 10, 807–823.
Wong, K., Sidransky, E., Verma, A., Mixon, T., Sandberg, G.D., Wakefield, L.K., Morrison, A., Lwin, A., Colegal, C., Allman, J.M., Schiffrmann, R., 2004. Neuropathology provides clues to the pathophysiology of Gaucher disease. Mol. Genet. Metab. 82, 192–207.
Yap, T.L., Gruschus, J.M., Velayati, A., Westbrook, W., Goldberg, E., Meaov, N., Sidransky, E., Lee, J.C., 2011. Alpha-synuclein interacts with Glucocerebrosidase providing a molecular link between Parkinson and Gaucher diseases. J. Biol. Chem. 286, 20808–20808.