Neurobiology of Disease xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Mitochondrial dysfunction associated with glucocerebrosidase deficiency

Matthew E. Gegg, Anthony H.V. Schapira *

Department of Clinical Neuroscience, UCL Institute of Neurology, London NW3 2PF, UK

ARTICLE INFO

Article history:
Received 27 May 2015
Revised 8 September 2015
Accepted 16 September 2015
Available online xxxx

Keywords:
Autophagy
Gaucher disease
Glucocerebrosidase
Lysosome
Mitochondria
mitophagy
Parkinson's disease

ABSTRACT

The lysosomal hydrolase glucocerebrosidase (GCase) is encoded for by the *GBA* gene. Homozygous *GBA* mutations cause Gaucher disease (GD), a lysosomal storage disorder. Furthermore, homozygous and heterozygous *GBA* mutations are numerically the greatest genetic risk factor for developing Parkinson's disease (PD), the second most common neurodegenerative disorder. The loss of GCase activity results in impairment of the autophagy-lysosome pathway (ALP), which is required for the degradation of macromolecules and damaged organelles. Aberrant protein handling of α-synuclein by the ALP occurs in both GD and PD. α-synuclein is the principle component of Lewy bodies, a defining hallmark of PD. Mitochondrial dysfunction is also observed in both GD and PD. In this review we will describe how mitochondria are affected following loss of GCase activity. The pathogenic mechanisms leading to mitochondria dysfunction will also be discussed, focusing on the likely inhibition of the degradation of mitochondria by the ALP, also termed mitophagy. Other pathogenic cellular processes associated with *GBA* mutations that might contribute, such as the unfolding of GCase in the endoplasmic reticulum, calcium dysregulation and neuroinflammation will also be described. Impairment of the ALP and mitochondria dysfunction are common pathogenic themes between GD and PD and probably explain why *GBA* mutations increase the risk of developing PD that is very similar to sporadic forms of the disease.

1. Glucocerebrosidase

Glucocerebrosidase (GCase; also known as glucosylceramidase; EC 3.2.1.45) is a lysosomal enzyme involved in sphingolipid metabolism. GCase catabolises the substrate glucosylceramide (GlcCer) to glucose and ceramide. Ceramide is the hydrophobic membrane anchor of all sphingolipids and is recycled to generate new glycosphingolipids (e.g. gangliosides such as GM1, GM2, sulfatide) and sphingomyelins (van Echten-Deckert and Herget, 2006).

GCase is encoded by the *GBA* gene located on chromosome 1q21 and is comprised of 11 exons, encoding for a protein of approximately 62 kDa. Upon translation, GCase is transported to the lysosome by binding to the transport receptor LIMP-2 in the endoplasmic reticulum (Reczek et al., 2007).

1.1. Gaucher disease

Autosomal recessive *GBA* mutations (homozygous or compound heterozygote) cause Gaucher disease (GD), the most common lysosomal storage disorder. Over 300 *GBA* mutations have been identified to cause GD (Hruska et al., 2008; Sidransky et al., 2009). Mutations lead to loss of GCase activity in the lysosome, resulting in the accumulation of GlcCer (Nilsson and Svennerholm, 1982; Nilsson et al., 1985). Accumulation of substrate in the lysosomes of macrophages is the main manifestation in visceral organs, leading to hepatosplenomegaly, anaemia, thrombocytopenia and bone involvement (Grabowski, 2008). While the above manifestations are common to all GD patients, GD is further classified in to non-neuronopathic (Type 1; OMIM#2308000) and neuronopathic (types 2 and 3; OMIM#23099 and OMIM 2301000, respectively). Onset of type 1 occurs in childhood or adulthood. Type 2 is the most severe, with substantial neurodegeneration and a median age of death at 9 months. Type 3 also has neurodegeneration, with death in childhood or early adulthood (Grabowski, 2008).

GBA mutations include point mutations, insertions, deletions, frame shifts, splice-site alterations and recombinant alleles, with patients showing considerable clinical heterogeneity despite similar genotypes. However, the mutant *N370S* allele, even in combination with another *GBA* mutant allele is predictive of Type 1 GD (Grabowski, 2008; Sun et al., 2013). Furthermore the *L444P* allele is strongly associated with neuronopathic GD, with a combination of *L444P* and another complex allele leading to type 2, and homozygous *L444P* mutations resulting in type 3. The *N370S* and *L444P* allele are the most common mutations associated with GD (Hruska et al., 2008).

Studies have shown that the intrinsic catalytic activity of N370S and L444P mutant GCase to be reduced by 80–95% compared to wild-type

* Corresponding author at: Department of Clinical Neuroscience, UCL Institute of Neurology, Hampstead Campus, Rowland Hill Street, London NW3 2PF, UK.
E-mail address: a.schapira@ucl.ac.uk (A.H.V. Schapira).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.09.006
0969-9961/

(Grace et al., 1999; Liou et al., 2006; Salvioli et al., 2005). Biochemical and molecular dynamic studies have suggested that N370S GCase is less able to associate with the physiological GCase activator saposin C and anionic phospholipids (Offman et al., 2010; Salvioli et al., 2005). However, loss of GCase activity is not solely due to impaired catalytic activity, but also a reduction in GCase protein levels. Many GCase mutations, including N370S and L444P, unfold in the endoplasmic reticulum (ER) at neutral pH, whereupon they are extracted by chaperones and degraded by the proteasome (Mu et al., 2008; Ron and Horowitz, 2005). This process is known as ER-associated degradation (ERAD).

1.2. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The loss of dopaminergic neurons in the substantia nigra results in movement disorders such as resting tremor, bradykinesia, rigidity and postural instability (Schapira and Jenner, 2011). In addition to neurodegeneration, PD is characterised by the presence of Lewy bodies in surviving neurons, intracellular protein inclusions predominantly consisting of α-synuclein. Mitochondrial dysfunction is also associated with PD pathogenesis (reviewed by Schapira and Gegg, 2011). Decreased activity of complex I of the electron transport chain (ETC) occurs in the substantia nigra of PD brains (Schapira et al., 1989). Several genes associated with early-onset autosomal recessive PD such as DJ-1, parkin and PINK1 have further strengthened the link between mitochondria and PD (Bonifati et al., 2003; Kitada et al., 1998; Valente et al., 2004). In particular, PINK1, a mitochondrial serine threonine kinase, and parkin, an E3 ubiquitin ligase, have recently been identified as key players in the identification and removal of damaged mitochondria by macroautophagy (Matsuda et al., 2010; Narendra et al., 2008; Vives-Bauza et al., 2010). The role of impaired autophagy in both PD and GD will be discussed further below.

1.3. GBA mutations and PD

In 1996 it was first reported that some type 1 GD patients exhibited typical Parkinsonism (Neudorfer et al., 1996). Further investigations then indicated that people with heterozygote *GBA* mutations were at greater risk of developing PD. A multicenter meta-analysis containing over 5000 PD patients reported the odds ratio of a PD patient carrying a *GBA* mutation to be 5.4 (Sidransky et al., 2009). Approximately 5–10% of PD patients carry a *GBA* mutation, making these mutations the most numerical genetic risk factor for developing PD. See Table 1 for a table summarising the main studies linking *GBA* with PD.

The age of onset of PD in patients with *GBA* mutations is approximately 5 years earlier than sporadic PD cases (Neumann et al., 2009; Sidransky et al., 2009). GBA-associated parkinsonism resembles sporadic PD, with no noticeable changes in Lewy body pathology reported either (Neumann et al., 2009; Parkkinen et al., 2011). However, cognitive impairment is more frequent in PD patients with GBA mutations (Alcalay et al., 2012; Beavan et al., 2015; Brockmann et al., 2011). It should be noted that *GBA* mutations are also significantly associated with developing dementia with Lewy bodies (DLB) and PD with dementia (odds ratios of 8.3 and 6.5, respectively; Nalls et al., 2013).

The two most frequent GBA mutations associated with PD are N370S and L444P, accounting for up to 17–31% of all PD patients in the European Ashkenazi Jewish population, and 3% in non-Ashkenazi populations (Neumann et al., 2009; Sidransky et al., 2009). Biochemical analysis of PD brains with *GBA* mutations indicated a significant decrease in GCase activity, with the greatest deficiency (58% decrease in enzyme activity) occurring in the substantia nigra (Gegg et al., 2012). Western blot analysis indicated that this loss of activity was in part due to a decrease in protein expression. Markers of the unfolded protein response (UPR) were increased in PD brains with GBA mutations, suggesting that mutant GCase is degraded by ERAD (Gegg et al., 2012).

Notably, GCase activity and protein expression was also significantly decreased by 33% in the substantia nigra of sporadic PD brains (Gegg et al., 2012). Another study indicated that GCase protein expression was significantly decreased in the anterior cingulate cortex of sporadic PD brains (Murphy et al., 2014). This study also showed that the decreased GCase protein levels in the brain significantly correlated with increased α-synuclein levels. This supports cell culture studies that had indicated that increased α-synuclein levels decreased GCase protein levels by inhibiting the trafficking of GCase to the lysosome (Gegg et al., 2012; Mazzulli et al., 2011).

1.4. Lysosomal dysfunction in GD and PD

The accumulation of GlcCer in lysosomes of GD patients and the well documented lysosomal dysfunction associated with PD (Alvarez-Erviti et al., 2010; Cuervo et al., 2004; Dehay et al., 2010), has meant that much of the GCase research in recent years has focused on the autophagy lysosome pathway (ALP). However it is important to note that the mechanisms leading to GD and GD patients developing PD are unlikely to be identical to PD patients with heterozygote *GBA* mutations. The greatest disparity is likely to be the accumulation of GlcCer and GlcSph in peripheral organs and brains of type 1, 2 and 3 GD patients (Nilsson and Svennerholm, 1982; Nilsson et al., 1985; Orvisky et al., 2002). Mouse models of neuronopathic GD models also have accumulation in the brain (Enquist et al., 2007; Farfel-Becker et al., 2014; Sun et al., 2013). However, no accumulation of GlcCer or GlcSph was detected in PD brains with heterozygote *GBA* mutations (Gegg et al., 2015), or mouse models lacking one of the *GBA* alleles (*GBA* +/−; Farfel-Becker et al., 2014; Sardi et al., 2011). Analysis of whole brain homogenates does not differentiate between neurons, glia and other cell types in the brain. A very modest accumulation of GlcCer has been reported in cortical mouse neurons with 50% decreased GCase activity following knock down (KD) with RNAi (Mazzulli et al., 2011)and in neurons differentiated from inducible pluripotent stem cells (iPSC) containing heterozygote *GBA* mutations (Schöndorf et al., 2014).

While it is still unclear exactly how *GBA* mutations impact on lysosomal function, it is evident that loss of GCase activity effects the ALP in both heterozygote and homozygote *GBA* models. Two processes of the ALP are particularly relevant to GCase deficiency: chaperone mediated autophagy (CMA) and macroautophagy (Fig. 1). CMA involves the degradation of soluble proteins containing a KFERQ pentapeptide motif. Unfolded proteins are delivered to the lysosome via the chaperone hsc70, and the proteins are then directly translocated in to the lysosome for degradation via the integral membrane protein LAMP2A (reviewed by Mizushima et al., 2008; Parzych and Klionsky, 2014). Macroautophagy also degrades macromolecules such as protein and lipids, but also has the capacity to degrade larger structures, such as aggregated proteins and damaged organelles like mitochondria (Mizushima et al., 2008; Parzych and Klionsky, 2014). Cargo for

Table 1
Summary of main studies linking GBA and PD.

Links between mutant GBA and PD	References
Subset of GD patients develop parkinsonism	Bembi et al. (2003), Neudorfer et al. (1996), Tayebi et al. (2001)
Genetic multi-centre study reporting statistically significant association between *GBA* mutations and PD	Sidransky et al. (2009)
Lewy bodies similar in PD brains with and without *GBA* mutations	Neumann et al. (2009), Parkkinen et al. (2011)
Accumulation and impaired turnover of α-synuclein in animal and cell models of GCase deficiency	Cullen et al. (2011), Fishbein et al. (2014), Manning-Boğ et al. (2009), Mazzulli et al. (2011), Osellame et al. (2013), Sardi et al. (2011), Schöndorf et al. (2014)
GCase activity decreased in sporadic PD	Gegg et al. (2012), Murphy et al. (2014)
α-synuclein affects GCase function	Gegg et al. (2012), Mazzulli et al. (2011), Sardi et al. (2013), Yap et al. (2011)

Please cite this article as: Gegg, M.E., Schapira, A.H.V., Mitochondrial dysfunction associated with glucocerebrosidase deficiency, Neurobiol. Dis. (2015), http://dx.doi.org/10.1016/j.nbd.2015.09.006