Pathological looping in the synucleinopathies: investigating the link between Parkinson’s disease and Gaucher disease

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Summary and comment on a recent Cell paper entitled ‘Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies’ (Mazzulli et al., 2011).

The close relationship between Gaucher disease (GD) and Parkinson’s disease (PD) is now well established. PD is the commonest neurodegenerative movement disorder and is characterised histopathologically by the selective loss of dopaminergic neurons in the substantia nigra, and clinically by tremor, bradykinesia, rigidity and postural instability. Most cases of idiopathic PD display bradykinesia, rigidity and postural instability. The close relationship between Gaucher disease and PD was recognised many years ago (Benstock et al., 1980) and was further supported by a number of large cohort studies in several different populations that have shown a greater frequency of GBA1 mutations in patients with PD (Neumann et al., 2009) [see also Sidek et al. (Sidek et al., 2009) and references therein]. Thus, heterozygous GBA1 mutations are now considered to be the most common genetic risk factor for PD as well as for related synucleinopathies, such as dementia with LBs (DLB).

GD is the most common lysosomal storage disorder and is caused by homozygous mutations in the glucocerebrosidase gene (GBA1), which encodes glucocerebrosidase (GCase; also known as glucosylceramidase). This enzyme mediates the lysosomal hydrolysis of glucosylceramide (GluCer) to form ceramide and glucose. Loss of GCase activity leads to lysosomal accumulation of GluCer and glucosylphosphoisine in a number of cell types, including macrophages and neurons, giving rise to the visceral and neurological manifestations of GD, respectively (Cox, 2010) [see review by Farfel-Becker et al. (Farfel-Becker et al., 2011) in this issue for a clinical classification of GD]. There are currently no therapies available that target the central nervous system pathology associated with GD, because existing treatments, such as recombinant enzyme replacement therapy, fail to permeate the blood-brain barrier (Cox, 2010).

One of the first clues that there might be a link between PD and GD arose from a small number of case reports describing parkinsonian features in GD patients (Neudorfer et al., 1996; Tayebi et al., 2003; Bembi et al., 2003). This was followed by the finding that parkinsonism occurs at an increased frequency in the relatives of GD patients who are carriers of GBA1 mutations (Goker-Alpan et al., 2004). Since these initial observations, there have been a number of large cohort studies in several different populations that have shown a greater frequency of GBA1 mutations in patients with PD (Neumann et al., 2009) [see also Sidek et al. (Sidek et al., 2009) and references therein]. Thus, heterozygous GBA1 mutations are now considered to be the most common genetic risk factor for PD as well as for related synucleinopathies, such as dementia with LBs (DLB).

A number of studies, including those in GD mouse models (Farfel-Becker et al., 2011), have explored the pathogenic mechanisms linking mutant GCase with α-syn neuropathology, and implicate both loss of GCase enzymatic function and toxic gain-of-function processes (Xu et al., 2011; Sardi et al., 2011). The prominent loss-of-function theories focus on altered lipid metabolism. Specifically, disordered GluCer metabolism is postulated to interfere with α-syn binding to plasma membranes or to directly affect its fibrillisation, leading to an accumulation of toxic α-syn species. Support for the gain-of-function hypothesis has arisen from the fact that PD-associated GBA1 mutations are usually missense mutations that are likely to give rise to a protein product (Neumann et al., 2009). In keeping with this is the finding that mutant GCase is present within a significant proportion of α-syn inclusions in PD and GD brains (Goker-Alpan et al., 2010). One hypothesis is that mutant GCase directly interferes with the cellular autophagic-lysosomal mechanisms that mediate α-syn degradation (Cuervo et al., 2004; Vogiatzi et al., 2008), resulting in intracellular α-syn accumulation and subsequent LB formation (Fig. 1).

The relative contributions of the enzymatic loss-of-function and toxic gain-of-function mechanisms to GBA1-associated PD have remained elusive. Mazzulli et al. studied the effects of loss of GCase activity on α-syn neurotoxicity using cell and mouse models, as well as human post-mortem data (Mazzulli et al., 2011). They first demonstrated that knockdown of GCase activity in neuronal culture caused loss of GCase enzymatic function and toxic gain-of-function processes (Xu et al., 2011; Sardi et al., 2011), have explored the pathogenic mechanisms linking mutant GCase with α-syn neuropathology, and implicate both loss of GCase enzymatic function and toxic gain-of-function processes (Xu et al., 2011; Sardi et al., 2011). The prominent loss-of-function theories focus on altered lipid metabolism. Specifically, disordered GluCer metabolism is postulated to interfere with α-syn binding to plasma membranes or to directly affect its fibrillisation, leading to an accumulation of toxic α-syn species. Support for the gain-of-function hypothesis has arisen from the fact that PD-associated GBA1 mutations are usually missense mutations that are likely to give rise to a protein product (Neumann et al., 2009). In keeping with this is the finding that mutant GCase is present within a significant proportion of α-syn inclusions in PD and GD brains (Goker-Alpan et al., 2010). One hypothesis is that mutant GCase directly interferes with the cellular autophagic-lysosomal mechanisms that mediate α-syn degradation (Cuervo et al., 2004; Vogiatzi et al., 2008), resulting in intracellular α-syn accumulation and subsequent LB formation (Fig. 1).

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They next showed that depletion of GCase in primary neuronal culture caused neurotoxicity when either wild-type α-syn or α-syn containing the PD-linked A53T mutation was overexpressed. Furthermore, they established that this neurotoxicity was
Loss of enzymatic function: pathogenic positive-feedback loop proposed by Mazzulli et al. (2011)

Toxic gain-of-function:
- Direct interaction between mutant GCase and α-syn?
- Inhibition of the lysosomal-autophagic degradation of α-syn by mutant GCase?

Fig. 1. Potential mechanisms linking mutant GCase with PD. Loss of enzymatic function owing to mutation might result in a pathogenic positive-feedback loop, as proposed by Mazzulli et al. (Mazzulli et al., 2011) (red arrows); alternatively, toxic gain-of-function effects might link mutant GCase with α-syn neurotoxicity.

dependent on the ability of the α-syn to polymerise, because removal of amino acids 71-82 [which are essential for α-syn fibrillisation (Giasson et al., 2001)] abolished α-syn neurotoxicity. In keeping with this required property of α-syn, GCase depletion was found to be associated with increased levels of high molecular weight oligomeric α-syn species, which are intermediates in the pathway to fibril formation.

By demonstrating that pharmacological lysosomal inhibition is insufficient to cause α-syn neurotoxicity in cell culture, the authors hypothesised that changes in GluCer metabolism might be responsible for the formation of α-syn oligomeric species. Indeed, lipid dispersions containing high concentrations of GluCer were able to stabilise α-syn in soluble oligomeric forms under lysosomal conditions. In particular, immunoelectron microscopy showed that the α-syn aggregates localised directly onto tubular structures formed by the GluCer mixtures. Mazzulli et al. then confirmed their findings in a triple-mutant GD mouse model that is homozygous for a GD-related GBA1 variant (V394L) and that also expresses a hypomorphic prosaposin gene (4L/PS-NA) (Farfel-Becker et al., 2011), to further reduce GCase activity (Xu et al., 2011; Mazzulli et al., 2011). These mice exhibit GluCer accumulation and α-syn build-up in the brain, which is associated with age-dependent neurological deficits (Xu et al., 2011). As expected, Mazzulli et al. found that soluble oligomeric forms of α-syn were present in these GD mouse brains and were particularly localised to areas of neurodegeneration (Mazzulli et al., 2011). Further in vivo evidence for the accumulation of α-syn in response to GCase depletion was also provided in the D409H mutant GCase mouse model (D409H/D409H) (Xu et al., 2011; Farfel-Becker et al., 2011) and in studies using Caenorhabditis elegans. These results are in line with previous work showing that pharmacological inhibition of GCase leads to the accumulation of α-syn in neuroblastoma cells and mouse models (Manning-Bog et al., 2009).

Having established that perturbations in GCase activity led to accumulation of toxic α-syn oligomers in vitro and in vivo, Mazzulli et al. then showed that these mechanisms are also relevant to the human disease (Mazzulli et al., 2011). Analysis of post-mortem brain samples from neuronopathic GD patients, and from patients with DLB, confirmed the presence of α-syn oligomers in association with reduced GCase activity.

Finally, the authors sought to address whether GCase plays a role in PD in the absence of a GBA1 mutation. The idea that α-syn might have reciprocal effects on GCase arose from recent work in mammalian cells and in yeast showing that α-syn can affect the trafficking of proteins from the endoplasmic reticulum (ER) to the Golgi by interfering with the formation of SNARE complexes (Cooper et al., 2006; Thayanidhi et al., 2010). It is therefore feasible that raised levels of α-syn in PD brains interfere with the lysosomal maturation and activity of GCase. To test these hypotheses, Mazzulli et al. first used primary cortical neurons and demonstrated that overexpression of either wild-type or A53T-mutant α-syn resulted in retention of GCase in the ER, associated with reduced lysosomal GCase activity. By studying GCase glycosylation patterns in PD and healthy control brain tissue, they then confirmed this in vitro data and showed that α-syn levels affect the ER-Golgi trafficking of GCase: higher levels of α-syn in the brain were correlated with reduced lysosomal GCase levels.

In summary, Mazzulli et al. demonstrated that depletion of GCase activity led to reduced lysosomal degradation and accumulation of α-syn, in association with GluCer build-up (Mazzulli et al., 2011). In turn, GluCer was able to stabilise soluble oligomeric α-syn species. They also found that increased α-syn levels reduced the lysosomal maturation and activity of GCase, suggesting that GCase plays a part in the pathogenesis of sporadic PD. Furthermore, they propose that these bidirectional effects between GCase and α-syn might form a self-
propagating positive-feedback loop in PD and other synucleinopathies (Fig. 1).

This paper offers considerable new insights into the complex interplay between GBA1 mutations and α-syn neurotoxicity in PD, and provides greater evidence for the theory that the neuropathology is caused by a loss of GCase function. It also suggests that rescuing lysosomal GCase activity, thereby breaking the positive-feedback cycle of α-syn accumulation, might be a viable treatment strategy for sporadic as well as GBA1-associated PD. This is also in line with a recent study by Sardi et al. (Sardi et al., 2011) in GD mice (D409V/D409V) (Farfel-Becker et al., 2011), showing that the delivery of GCase to the brain by gene therapy rescues the α-syn-mediated pathology and associated cognitive deficits.

Of course, this positive-feedback-loop theory also poses new questions. Although it offers an insight as to how small increases in α-syn in the cell can build to harmful levels, this theory does not explain fully why only a minority of GD patients or carriers of GBA1 mutations ever develop PD. Therefore, other pathological factors are probably involved that determine whether the positive-feedback loop can surpass the cellular homeostatic mechanisms to an extent that the levels of α-syn become toxic. For example, other genetic variants associated with PD or an age-dependent decline in cellular degradative capacity might tip the balance in favour of pathological α-syn accumulation and neurotoxicity in affected individuals. Moreover, mutant GCase has been shown to promote α-syn accumulation in cellular and mouse models independently of its reduced enzymatic activity (Cullen et al., 2011; Sardi et al., 2011), and Yap et al. have recently shown that GCase and α-syn can directly interact under lysosomal solution conditions (Yap et al., 2011). These observations support the hypothesis that both loss of enzymatic GCase function and toxic gain-of-function effects probably act in concert to provoke α-syn neurotoxicity associated with GBA1 mutations. Further studies will be required to test this positive-feedback-loop theory, and to identify other contributing factors that might be involved in driving this pathway towards the diseased state, in order to understand fully the link between GD and PD. New and improved mouse models of GD will undoubtedly aid this process, leading to the development of new therapies that successfully cross the blood-brain barrier and ameliorate the central nervous system disease.

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REFERENCES