Genetics provides new individualized therapeutic targets for Parkinson's disease

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The identification of genes increasing one's risk of developing common, complex disorders like Parkinson's disease (PD) can provide novel therapeutic opportunities. A prime example of this are the consequences of mutations in *GBA1*, the gene responsible for the lysosomal storage disorder Gaucher disease (GD). GD is a multi-system disorder, primarily affecting tissues of the reticuloendothelial system. A subset of patients with GD also have neuronopathic manifestations (types 2 and 3 GD). In addition, there is an established association between GD and seemingly-unrelated movement disorders. First identified through clinical studies in patients and families with GD, mutations in *GBA1* are the most significant genetic risk factor for PD and associated neurodegenerative disorders, including dementia with Lewy bodies and rapid eye movements sleep behavior disorders (Sidransky et al., 2009; Sidransky and Lopez, 2012; Honeycutt et al., 2019). This discovery has directed increased attention to lysosomal dysfunction in PD pathogenesis, rendering glucocerebrosidase (GCase), the enzyme encoded by *GBA1*, an attractive target for therapeutic development, as recently reviewed by Chen et al. (2020).

The mechanisms underlying the association between mutations in *GBA1* and parkinsonism are still not fully understood. Different hypotheses have been reviewed extensively elsewhere (Blandini et al., 2019; Do et al., 2019; Avenali et al., 2020). However, there appears to clearly be an inverse relationship between levels of glucocerebrosidase and the accumulation of alpha-synuclein. Furthermore, enzymatic studies of patients with PD reveal that even those without *GBA1* mutations have reduced levels of glucocerebrosidase. Thus, therapies that increase glucocerebrosidase may have broad implications for the treatment of parkinsonism in general.

While 4–20% of patients with PD, depending on ethnicity, carry mutations in *GBA1*, the penetrance is very low. Interestingly, patients with PD carrying *GBA1* mutations have an earlier age-of-onset and faster disease progression of parkinsonian manifestations. However, a majority of *GBA1* mutation carriers, even those with close family members with PD, do not develop parkinsonism (Sidransky and Lopez, 2012). Thus, other genetic and/or non-genetic modifiers may impact whether *GBA1* mutation carriers eventually go on to develop PD. Identifying these secondary risk factors, or "modifiers," is particularly challenging, but these modifiers can have great significance and may potentially identify additional pathways or regulators that may serve as effective therapeutic targets (Davidson et al., 2018).

Enzyme replacement therapy, the first effective treatment for GD, reverses the hallmark symptoms of anemia, thrombocytopenia, and hepatosplenomegaly. Initially derived from placenta, several approved forms of the recombinant enzyme are now available. Though enzyme replacement therapy ameliorates the visceral phenotypes observed in patients, it does not effectively treat the neurological dysfunction in neuronopathic forms of GD due to its failure to cross the blood-brain barrier. Similarly, substrate reduction therapy, which reduces the synthesis of the stored sphingolipids, is ineffective in reversing neuronopathic symptoms (Bennett and Mohan, 2013). Given that *GBA1* mutation carriers are at an increased risk of developing PD, novel therapeutics are needed to effectively treat the parkinsonian manifestations in patients with *GBA1*- associated PD, and potentially, in other patients with PD.

To address this issue, multiple therapeutic options have focused on the delivery of GCase to the brain using several different methods. A study performed by Massaro et al. (2018) demonstrated that fetal intracranial injection of an adeno-associated virus serotype 9 (AAV9) vector with the *Gba1* gene reconstituted neuronal GCase expression in a mouse model of neuronopathic GD. Long-term assessments of efficacy of intracerebroventricular and intravenous gene therapy showed that treated neuronopathic GD mice survived at least 180 days and were indistinguishable from age-matched controls. A similar study conducted by Rocha et al. (2015) demonstrated the effectiveness of intracerebral injection of AAV-GBA1 vector in a mouse model over-expressing A53T alpha-synuclein in reducing insoluble a-synuclein aggregates in the substantia nigra and striatum. AAV vectors provide an attractive mode of delivery due to their non-integrative nature and its selective delivery to the central nervous system.

Pharmacological small-molecule chaperones serve as another attractive approach to treating patients with *GBA1*-associated PD. Since they have the ability to cross the blood-brain barrier and directly bind to mutant GCase, these chaperones are prime candidates for ameliorating the neurodegeneration seen in *GBA1*-associated PD and potentially idiopathic PD. Inhibitory chaperones have been investigated as GCase chaperones. A recently identified inhibitory chaperone, Ambroxol has been shown to increase neuronal GCase activity in both mice and non-human primates. Several clinical trials of Ambroxol are underway aiming to evaluate its effectiveness in improving the molecular dysfunction resulting from GCase deficiency and the neuromotor impairment observed in PD. However, the search continues to identify other chaperones that also do not have an inhibitory component. As explored in the review by Chen et al. (2020), efforts were focused on screening for non-inhibitory chaperones by performing a high-throughput screen of 250,000 compounds using tissue samples derived from a GD patient as the source of mutant enzyme. This yielded two non-inhibitory compounds, NCGC607 and NCGC758, that were further studied in midbrain dopaminergic neurons generated from patient-derived iPSCs. Both compounds have demonstrated increases in enzyme levels and activity, reduction of GCase substrates and the ability to lower alpha-synuclein levels and remove alpha-synuclein aggregates, respectively.

Although targeting the GCase enzyme is imperative for existing and future GD therapeutic development, strategies focusing on proteins that modify the disease progression can also be pursued to discover novel and efficacious therapeutics. GD, despite being a Mendelian disorder, is hallmarked by vast phenotypic heterogeneity. It is often difficult to use genotype-phenotype correlation to explain the various visceral and neuronopathic manifestations observed in patients. It seems certain that GD has other "modifiers" that modulate disease progression. Furthermore, the association of GD and PD further complicates the picture, for both homozygous and heterozygous *GBA1* mutation carriers have an increased PD risk. Identifying disease modifiers in a rare disorder is particularly challenging. However, a few candidate modifiers of GD have been identified, including *PSAP* and *SCARB2*, as explored in a review by Davidson et al. (2018).

PSAP, or prosaposin, is cleaved to form its by-product, Saposin C (Sap C). As an activator of GCase activity, Sap C has been shown to modulate some of the disease symptomology of GD. Mice deficient in both *Gba* and *Psap* exhibited a greater disease burden when compared to mice who were deficient in only one of the two proteins (Sun et al., 2010). Additionally, chemically synthesized Sap C increased GCase activity and stabilized GCase (Yoneshige et al., 2015). By combining Sap C and GCase treatments, current therapies might be improved to increase efficacy. A recent study by Oji et al. (2020) also suggests that variants in another by-product of PSAP, Saposin D (Sap D), are associated with PD. Pathogenic variants in the Sap D domain of PSAP were identified

in patients with autosomal dominant PD and several intronic variants were described as a susceptibility region for sporadic PD. Despite the rarity of mutations in the Sap D domain of *PSAP* and the conclusion that the contribution of Sap D to PD pathology is independent of sphingolipid hydrolase deficiencies, the results of the study implicating Sap D domain mutations in PD alpha-synuclein aggregation and loss of dopaminergic neurons point to the utility of identifying modifiers for developing targeted therapeutics.

SCARB2, encoding for the GCase transporter LIMP-2, has also been identified as a potential modifier of GD. Proper GCase trafficking is dependent on the pivotal interaction between LIMP-2 and GCase, with LIMP-2 deficient mice models exhibiting reduced GCase activity and expression. Mutations in *SCARB2* results in different disorders with neurological manifestations, including neurodegeneration and alpha-synuclein accumulation, and has been implicated in myoclonic epilepsy. The accumulation of alpha-synuclein seen is of particular interest, suggesting that *SCARB2* might be pursued to address parkinsonian symptoms in *GBA1*-associated PD.

In addition to these two GCase-related proteins, the use of newer genomic strategies may elucidate further disease modifiers that contribute to phenotypic heterogeneity in GD (Davidson et al., 2018). Linkage analysis, CRISPR/RNAi screens, and genome-wide association studies were all posited as helpful approaches to identify genetic modifiers. Furthermore, "epigenetic modifiers" could also contribute to discordant GD phenotypes (Hassan et al., 2017). In GD, with discordant sib-pairs and even discordant monozygotic twins, gene variants alone cannot fully explain the disparity of symptoms. The inclusion of both exogenous and endogenous stimuli impacting the dysregulation of the epigenetic mechanisms in *GBA1* mutation carriers add another layer of intricacy complicating the search for non-GCase modifiers that may serve as therapeutic targets.

Through the development and availability of various "omics" technologies, distinguishing the most probable candidate modifiers is now becoming a feasible task. Whole-genome sequencing and whole-exome sequencing provide platforms to identify significant intronic and exonic variants that could contribute to the development of PD in family and large-cohort studies (Bandres-Ciga et al., 2020).

Microarrays and RNAseq data can contribute transcriptomic insights based on differentially expressed genes to distinguish biologically significant pathways that can aid in finding probable modifiers. To investigate epigenomic contributions to GD and even *GBA1*-associated PD progression, DNA methylation arrays, small RNA sequencing, and ChIP-seq could be employed. Using mass spectrometry, proteomics analyses could aid in identifying other protein modifiers like *PSAP* and *SCARB2*. Similarly, genetic and environmental contribution to metabolomics and their downstream effects in *GBA1* mutation carriers could be determined by employing mass spectrometry and nuclear magnetic resonance spectroscopy. Recent advances in single cell technologies present a unique opportunity to identify modifiers in not only tissues, but in specific cellular populations as well. From scRNA-seq to scATAC-seq, numerous existing "omics" technologies can be expanded upon with greater resolution for more focused investigations of interactions with GCase and modifiers. However, it will be the careful and deliberate integration of all of these various "omics" platforms which will allow for powerful and effective analysis for the most plausible candidate modifiers for GD and *GBA1*-associated-PD.

Targeting and replenishing GCase has proven to be effective in treating patients with GD. However, the inability for enzyme replacement therapy and substrate reduction therapy to reverse neuronopathic symptoms continues to be an obstacle in treating neuronopathic GD and *GBA1*-associated PD. The possibility of targeting proteins other than GCase may eventually provide alternative avenues to treating PD symptoms and neuronopathic manifestations. In the era of personalized medicine, it becomes even more imperative to approach treatment of patients with GD and those with PD using a holistic strategy (Bandres-Ciga et al., 2020). Though further research is needed before effective treatments and therapeutics can be established, identifying modifiers of GD and its association with PD may prove to be a practical starting point. Ultimately, these modifiers of GD and GBA1-associated PD could provide novel insights regarding disease progression and, most importantly, other targets for developing therapeutics.

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