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Commentary

New Antibodies to Advance Glucocerebrosidase Research

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A most promising therapeutic target for the treatment of Parkinson's disease (PD) is the lysosomal hydrolase, glucocerebrosidase (GCase). Common variations in the *GBA1* gene encoding GCase are strongly associated with PD risk, and a number of identified mutations are associated with reduced GCase catalytic activity and subsequent accumulation of GCase lipid substrates [1, 2]. There is also evidence for reduced central and peripheral GCase activity in sporadic PD [3, 4], and a strong association occurs between reduced GCase activity, lysosomal dysfunction and accumulation of the hallmark pathological PD protein alpha-synuclein [5]. Consequently, there is great interest in therapeutic approaches to stabilize or modulate the activity of GCase as a potential way to treat PD. To go hand-in-hand with the development of novel therapeutic approaches targeting GCase, the development of research tools to assess target engagement is needed. Although it remains technically challenging, tools and methods to assess GCase activity and GCase lipid substrates are available [6]. In contrast, tools to robustly assess the levels and/or localization of the GCase protein itself have been difficult to establish. Previous studies have identified GCase antibodies that are reliable for immunoblot of human GCase protein under denaturing conditions [3, 7], yet robust assays for more quantitative measurement of GCase protein, particularly under native conditions, are not currently available. Excitingly however, in the manuscript titled "Characterization of novel human β-glucocerebrosidase antibodies for Parkinson disease research" in this issue of the Journal of Parkinson's disease, Jong and colleagues at the National Institutes of Health, in collaboration with Roche Pharma, report on the generation of new antibodies against human GCase that may now overcome this limitation [8].

A new series of monoclonal antibodies was generated using imiglucerase, the recombinant human GCase used in enzyme replacement therapy for patients with Gaucher disease (a lysosomal storage disorder caused by biallelic mutations in the *GBA1* gene). Hydbridoma clones were then screened against recombinant mouse and human GCase for immunoblotting and immunofluorescent applications, resulting in the selection of two clones termed hGCase-1/17 and hGCase-1/23. Although neither of the new clones could ultimately detect endogenous levels of GCase via immunoblot, they both demonstrated strong capability to detect GCase via immunostaining. Importantly, the specificity of the antibodies was confirmed using human cells deficient in the GCase protein. Moreover, using appropriate controls, the authors could further demonstrate that the new antibodies also worked for immunoprecipitation, and for the development of an AlphaLISA assay, opening new research avenues to interrogate GCase

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function and to quantify levels of GCase protein in a way not previously possible. The field will also be advanced by the making of these new tools available via the Michael J Fox Foundation for Parkinson's Disease Research.

These new tools represent a sizeable advance in the ability to measure and understand the GCase protein. Immunoprecipitation studies to measure GCase binding partners and post-translational modifications are now enabled, as are cell imaging studies to understand GCase subcellular localisation. It will also be exciting to explore the potential utility of these new antibodies in additional applications used in the field such as immunohistochemistry and flow cytometry, and to employ these new tools to better understand the link between GCase protein levels and activity and how this link is impacted by PD risk mutations and by sporadic PD. One drawback is that the newly generated antibodies were unable to detect mouse GCase for any of the assessed applications, and the specificity of the new antibodies for other preclinical species is still to be explored. Mapping of the epitope(s) to which the new antibodies bind could help understand species specificity and may also be important to determine how the numerous mutations and post translational modifications that occur across the GCase protein may affect antibody binding. In this regard, it is noted that the N370S GCase mutation that is commonly associated with PD was still recognized, but for further clinical development it may be important to know if mutations in particular regions of the GCase protein reduce antibody binding, and also if the two selected clones are recognising distinct or overlapping epitopes. Regardless, it will certainly be of interest to see the impact that these new reagents will have on the field as therapeutics targeting GCase continue through the development pipeline and into clinical trials.

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