Ectopic expression of human Glucocerebrosidase mutations in *Drosophila melanogaster* induces an Endoplasmic Reticulum stress response

The *GBA* gene encodes a hydrolytic lysosomal enzyme, glucocerebrosidase (GCase), responsible for breaking down glucosylceramide to ceramide and glucose. Homozygous mutations in *GBA* are known to cause Gaucher’s Disease (GD) whilst heterozygous mutations in *GBA* are the most frequently observed genetic risk factor for Parkinson’s disease to date. However, the detailed mechanism through which *GBA* influences the neurodegeneration seen in Parkinson’s disease (PD) is not yet clear. It is thought that the mutant GCase disrupts the degradation of α-synuclein and mitochondria through disruption of the autophagy–lysosomal pathway. Additionally, it is believed that the mutant GCase becomes trapped in the endoplasmic reticulum (ER), inducing the unfolded-protein response (UPR) and ER-associated degradation. α-synuclein build up is thought to exacerbate this process further by interfering with the binding between lysosomal membrane protein 2 (LIMP-2) and GCase, further disrupting GCase transport to the lysosomes.

To investigate these pathogenic mechanisms we used *Drosophila* lines engineered to express wild-type human *GBA* (*hGBA*), and two of the most common pathogenic *GBA* mutations associated with an increased risk of PD, N370S and L444P. We used the UAS-GAL4 system in *Drosophila* to overexpress the *GBA* variants in larval salivary glands and determined *GBA* co-localisation with the ER, using an ER marker fused to GFP and antibodies against both the GFP and *GBA*. We saw increased co-localisation in the mutant lines compared to the wild-type line and the control. Both the L444P and the N370S mutant lines additionally showed aggregations of *GBA* in the ER, although this was more pronounced in the L444P mutants. To determine if these aggregations are associated with an increased ER-stress response, we looked at the effect of expressing wild-type and mutant *hGBA* in the larval eye disc using a reporter of ER stress, Xbp1-EGFP. We quantified Xbp1-EGFP expression across all the lines using image analysis software and saw an increased expression in the mutant lines compared to the wild-type line and the controls. These data suggest that expression of mutant *hGBA* causes increased ER stress in *Drosophila*. Finally we tested whether we could rescue this ER stress using two drugs, Ambroxol and Isofagomine. These are FDA-approved molecules that act as pharmacological chaperones and enhance the stability and trafficking of mutated GCase (Maegawa GH, *et al*. 2009) (Richter F, *et al*. 2014). We observed a reduction in the elevated Xbp1-EGFP levels caused by the L444P expression with both drugs in our preliminary trials, although for Ambroxol a much more effective rescue was seen. Based on these data there is potential that in the future these drugs could be used as a treatment to reduce the ER-stress produced by the accumulation of mutant GCase molecules in both GD and PD patients.
