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International Conference on Glycobiology- The contribution of mutant GBA alleles to the development of Parkinson's disease in carriers of Gaucher disease mutations

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Abstract

Gaucher disease (GD) is an autosomal recessive disease resulting from mutations in the acid β-glucocerebrosidase (GCase) encoding gene, GBA, which leads to accumulation of glucosylceramide. GD patients and carriers of GD mutations have a significantly higher propensity to develop Parkinson's disease (PD) in comparison to the non-GD population. This implies that mutant GBA allele is a predisposing factor for development of PD in carriers of GD mutations. We have previously shown that in cells that derived from patients of GD and carriers of GD mutations, mutant GCase molecules undergo ER retention, which leads to lead to ER stress and to activation of the unfolded protein response (UPR). We used Drosophila melanogaster to confirm that development of PD in carriers of GD mutations results from the presence of mutant GBA alleles. Drosophila has two GBA orthologs and each one of them has a mutant allele. We generated two different Drosophila models for carriers of GD mutations: Flies double heterozygous for the two-endogenous mutant GBA orthologs and flies expressing the human N370S or the L444P GCase variants. All lines exhibited UPR, death of dopaminergic cells, shorter life span and had a decreased negative geotaxis. ER stress and Parkinsonian signs could be rescued by growing the double heterozygous flies or flies containing the N370S or the L444P mutant GCase variants in the presence of the pharmacological chaperone ambroxol. Our results strongly suggest that the presence of a mutant GBA allele in dopaminergic cells leads to ER stress and to their death and contributes to development of Parkinson's disease.

Gaucher disease (GD) is a lysosomal storage disease that results from mutation in the GBA gene encoding lysosomal acid β-glucocerebrosidase (GCase). The disease is characterized by accumulation of glucosylceramide mainly in monocyte-derived cells. Due to its heterogeneity, the disease has been divided into three types: the type 1 GD, primarily a non-neurological disease, and Type 2 and 3, two forms associated with a neuronopathic disease .More than 300 mutations were identified in the GBA gene. A large fraction of them are missense mutations, though premature termination, splice site mutations, deletions and recombinant alleles have been recognized as well. There are several abundant mutations. For example, the N370S mutation is the most prevalent among type 1 GD patients, while the L444P mutation is most common among the neuronopathic forms of GD. The majority of patients homozygous for the L444P mutation develop type 3 GD. The 84GG mutation is an insertion of a guanine 84 nucleotides downstream from the first initiator methionine of the GBA mRNA, resulting in premature protein termination.As a lysosomal enzyme, GCase in synthesized in the endoplasmic reticulum (ER) on ER-bound polyribosomes. Upon its entry into the ER, it undergoes N-linked glycosylation on four asparagines, after which it is subject to ER quality control. When correctly folded it shuttles to the Golgi compartment for further modifications on its N-linked glycans and finally it traffics to the lysosomes. Mutant GCase variants undergo ER-associated degradation (ERAD), the degree of which correlates with disease severity. ER-retained mutant GCase leads to ER stress and to unfolded protein response (UPR).In recent years, a large number of independent studies documented association between GD and Parkinson disease (PD). GD patients have significantly higher propensity to develop PD than non-GD population, and a high frequency of GD mutations were found in PD population . PD is the second most common motor neurodegenerative disorder that usually affects individuals above the age of 60. The hallmark of PD is the loss of dopaminergic cells in the substansia nigra pars compacta, which results in movement disorder with resting tremor, stiffness, postural instability and bradykinesia .The accumulation of misfolded proteins in the brain is a common feature in many neurodegenerative diseases such as Huntington's disease, Alzheimer's disease and Amyotrophic Lateral Sclerosis . Accumulation of misfolded proteins has also been documented in PD, contributing to ER stress and upregulation of the UPR pathways . This raises the possibility

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that accumulation of misfolded proteins in PD triggers stress-response pathways that induce neurotoxicity and cell death exhibited in brains of afflicted patients.As ERAD and UPR are well conserved across species, Drosophila has proven an excellent organism to model neuronal degenerative diseases in general and PD in particular (30–33). Deregulation of gene expression can be achieved in the fly by the use of existing mutations, the use of lines containing endogenous genes with an insertion of a transposable element (34), by siRNA or by mutating the gene using the CRISPR-Cas system (35,36). Transgenic expression of heterologous genes is another way to follow the in vivo effect of expression of normal or mutant genes in the fly. Expression of the foreign gene is regulated by the GAL4 transcription factor, under any desired promoter, with a wide or a very narrow expression pattern.In a previous study, we have shown UPR activation in fibroblasts derived from GD patients and in fibroblasts heterozygous for various GD mutations. We, and others, have also demonstrated that in Drosophila melanogaster models for carriers of GD mutations, UPR was activated and that locomotion of the flies was impaired .

In this study, we extended our analyses to show that aging flies, double heterozygous for the fly GBA variants, presented death of dopaminergic cells, impaired negative geotaxis and a shorter life span compared with normal flies. Transgenic flies expressing the N370S, L444P or the 84GG human mutations also showed UPR and developed parkinsonian signs. With the exception of the 84GG line, in all fly models UPR as well as parkinsonian signs could be partially rescued by the addition of the pharmacological chaperone ambroxol, highlighting the importance of misfolded protein in development of PD.

Antibodies for western blotting

The following primary antibodies were used in this study: rabbit polyclonal anti-TH antibodies AB152 (Millipore, MA, USA), rabbit polyclonal anti-phospho-eIF2α (Ser51) antibodies, rabbit polyclonal anti-eIF2α antibodies (from Cell Signaling Technology, Beverly, MA, USA), and mouse monoclonal anti-actin antibody (Sigma-Aldrich, Israel).Secondary antibodies used were: Horseradish peroxidase-conjugated goat anti-mouse antibodies and Horseradish peroxidase-conjugated goat anti-rabbit antibodies (both from Jackson Immuno Research Laboratories, West Grove, PA, USA).

Ambroxol treatment

Ambroxol (Sigma Aldrich, Rehovot, Israel) was added into 10 ml food containing vials, to a final concentration of 1 mM

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