Identification of modifiers of alpha-synuclein inclusion in a C. elegans model by genome-wide RNAi

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2.313 Alcoholism, alpha-synuclein, and Parkinson disease: A case-control study

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Objective: Variability in the allele length of a dinucleotide repeat sequence (REP1) within the alpha-synuclein gene (SNCA) promoter is associated with Parkinson's disease (PD) [JAMA 2006; 296: 661–670] and also with alcohol dependence [Hum Mol Genet 2005; 14: 967–971]. Medical diagnosis of alcoholism is inversely associated with PD [Neurology 2000; 14: 1350–1358]. The aim of this study was to determine whether the association of REP1 genotype and PD is independent of alcoholism, whether the association of alcoholism and PD is independent of REP1 genotype, and whether REP1 genotype and alcoholism have joint effects on PD susceptibility.

Method: Cases were recruited prospectively from the Department of Neurology of the Mayo Clinic in Rochester, MN, after June 1, 1996. The controls included unaffected siblings of cases and unrelated population controls. We assessed alcohol use using a structured telephone interview. We screened for alcoholism (overt or covert) using the CAGE questionnaire. Genotyping was performed using an ABI 3730 platform. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using conditional logistic regression models.

Results: Our study included a total of 893 case-control pairs. There was an increased risk of PD with increasing SNCA REP1 bp length (OR 1.18 for each REP1 genotype score unit, 95% CI 1.02–1.35; $p = 0.02$). There was a trend of decreasing risk of PD with increasing CAGE score ($p = 0.0002$). The association of REP1 genotype score with PD remained significant after adjusting for CAGE score, and the inverse association of CAGE score with PD remained significant after adjusting for REP1 genotype score. No significant pairwise interactions were observed for REP1 genotype and CAGE scores.

Conclusion: Alcoholism (as measured by CAGE score) and REP1 genotype score were associated with PD susceptibility (main effects), but they did not have multiplicative joint effects on PD susceptibility.

2.314 Identification of modifiers of alpha-synuclein inclusion in a *C. elegans* model by genome-wide RNAi

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Objective: The protein alpha-synuclein is tightly connected to development of Parkinson's disease: hallmark of PD is the presence of protein inclusions in the brain containing the protein alpha-synuclein. In addition, multiplication of the alpha-synuclein gene has been shown to be causative for PD. We aim to identify genes involved in the formation of alpha-synuclein inclusions to provide an understanding of the mechanism of and genetic susceptibility to age-related sporadic PD.

Method: We created nematodes (*C. elegans*) transgenic for human alpha-synuclein fused to yellow fluorescent protein to visually track inclusion formation. We used this model in a genome-wide RNAi feeding screen to find genetic inactivations that alter α-synuclein inclusion formation.

Results: Alpha-synuclein inclusions develop during aging in the transgenic *C. elegans* strain. The inclusions contain mostly mobile protein material and also immobile, aggregated protein at old age. Using genome-wide RNAi we identified genes that increase the formation of inclusions when silenced by RNAi. Interestingly, there was a significant overrepresentation of the genes that function in the ER, Golgi and vesicular membranes, when compared to random sets of genes. In addition, we found several genes associated with aging to increase inclusion formation when silenced.

Conclusion: We developed a small animal model of alpha-synuclein accumulation, in which age-dependent inclusion formation can be visually tracked. Using this model we found genes that increase the number of alpha-synuclein inclusions when silenced. The overrepresentation of genes with a role in vesicular trafficking and ER protein quality control functioning in the endomembrane system suggests that these processes are involved in the age-dependent formation of alpha-synuclein inclusions. The genes found may provide an understanding of genetic susceptibility to PD and molecular targets for therapy.

2.315 Identification of potent small molecule inhibitors of alpha-synuclein aggregation in cell culture and by in vitro screening

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Objective: A rotenone-induced cell culture model of Parkinson's disease (PD) was primarily utilized to test the ability of ProteoTech's small molecule library of compounds (representing new chemical entities) to inhibit aggregation of alpha-synuclein, a major component of PD Lewy bodies.

Method: In this cell culture model, A53T mutant alpha-synuclein was overexpressed in human BEM-17 neuroblastoma cells. Cells exposed to 1 or 5uM rotenone accumulated alpha-synuclein aggregates containing a large amount of beta-sheet secondary structure as detected by positive Thioflavin S fluorescence and quantitative image analysis.

Results: Treatment of rotenone-treated cells with different novel small molecule compounds identified 4 compounds (referred to as PD-61, 86, 31 and 13) that profoundly reduce the accumulation of Thioflavin-S positive alpha-synuclein aggregates by 87–91%, 73–91%, 40–84% and 57–70%, respectively. A marked reduction of alpha-synuclein aggregation by these compounds was confirmed using Thioflavin T fluorometry, Congo red binding assays and circular dichroism spectroscopy. In addition to their potent alpha-synuclein anti-aggregation properties, these compounds also provided good protection against rotenone-induced neurotoxicity in cell culture and in a *C. elegans* survival assay.

Conclusion: These studies indicate that we have identified unique small molecule compounds that have potent alpha-synuclein anti-aggregation and neuroprotective properties and thus may serve as promising new therapeutics for PD and related disorders.

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2.316 6-Hydroxydopamine nigrostriatal lesion modulates alpha-synuclein expression in different rat brain regions

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Objective: alpha-Synuclein is considered to be involved in pathogenesis of PD and other neurodegenerative diseases. Its filamentous aggregates are found as principal constituents of Lewy bodies and some others pathological intraneuronal deposits. Due to the localization of alpha-synuclein in presynaptic terminals, its role in synaptic function and plasticity was postulated as well. 6-Hydroxydopamine (6-OHDA) rat model of PD is characterized by acute loss of dopaminergic neurons, but specific alpha-synuclein aggregates were not reported. Therefore, this model could reveal possible role of alpha-synuclein in plasticity given that some compensatory sprouting of dopaminergic neurons exists after period of extensive neuronal death.

Method: Adult male Mill-Hill Hooded rats ($n = 24$) received four-site intrastriatal stereotaxic injection of 6-OHDA or saline (sham operated animals) into the right lateral striatum. Five weeks later, animals were killed by decapitation and the model was verified by tyrosine hydroxylase (TH) imunohistochemistry. Western blot analysis was used to detemine the level of alpha-synuclein protein and confocal microscopy for analysis of alpha-synuclein co-localization with TH, synaptophysine, MAP 2 and GAP-43.