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Lysosomal dysfunction as determinant of Parkinson's disease: investigating the potential role of novel risk genes

Introduction

Parkinson's Disease (PD), the second most common neurodegenerative disorder is characterised by the progressive loss of dopaminergic neurons in the Substantia Nigra. PD is genetically heterogenous, and only around 10% of PD cases has been assigned to a genetic cause. A cellular process becoming seen as central to PD pathology is the lysosomal degradation pathway. Dysfunction of lysosomal degradation impairs cellular homeostasis through decreased removal of damaged cellular content and it is thought that a dysregulation in these pathways enable progression of PD pathology. Interestingly, recent genome wide studies identified numerous risk genes with potential lysosomal function that could contribute to the development of PD (Table 1). To date, however, we have little information on how and whether the affected genes could contribute to PD. Therefore studying genes governing the lysosomal degradation pathway would benefit the understanding of PD progression and revealing novel therapeutic targets.

Aims

The purpose of this study was to investigate the potential pathogenic role of candidate PD risk genes involved in the lysosomal degradation pathway, that have been nominated by Genome Wide Association studies as potential cause of PD. To do this, the powerful model organism *Caenorhabditis elegans* has been used with genetic knockout models for these genes. I have studied dopamine-dependent physiology and lysosomal degradation pathway to identify the dysfunction of which candidate gene leads to PD pathology in *C. elegans.*

Techniques used

Behavioural analysis of dopaminergic function in C. elegans: Basal Slowing Assay: Dopamine is a conserved neurotransmitter between humans and *C. elegans*. In *C. elegans* it drives specific behavioural responses that can be used to assess the functionality of dopaminergic neurons. I have used the 'Basal Slowing' assay, which measures a slowing response of the worms when reaching their food source (*E. coli* OP50), a behaviour that is entirely dependent on dopaminergic neuronal signalling in well-fed worms.

Detecting steady-state levels of p62, an autophagy receptor protein that degrades via autophagy: Total protein lysates were used for detecting the steady state levels of the p62 orthologue protein (SQST-1) in *C. elegans* upon deletion of candidate lysosomal genes, the orthologues of which have been nominated as potential PD risk factors. p62 (SQST-1) is an autophagy receptor protein that recognizes and targets proteins to autophagy and being bound to the target p62 is also degraded in the autophagosome-lysosome degradation pathways. Therefore, increased level of p62 signals dysfunction of the lysosomal degradation pathway. I have used Western-blotting technique with C. elegans specific anti-p62 antibody (1:4000 dilution) to monitor p62 (SQST-1) protein level and mouse specific anti-Actin antibody (1:5000 dilution) as loading control.

Results and Outcomes

This study has identified ASAH1 (*asah-1* in C. elegans) as a strong candidate risk factor for developing PD. A dopamine-dependent behavioural assay, called basal slowing, has been performed on 11 deletion mutant worm strains of lysosomal genes along with the wild-type (N2(WT)) and *cat-2* mutant strains. *cat-2* mutants do not express dopamine as they lack tyrosine-hydroxylase, an enzyme involved in domapine synthesis (Table 1). Wild-type worms (WT) decrease their speed when reaching food (bacteria) on the maintenance plates as food acts as mechanical signal that is sensed by dopaminergic neurons in the worms. The mean speed decreased in WT worms with 49.16% while the *cat-2* mutant showed no basal slowing response (negative control). *ser-1* mutant lacking serotonin neurotransmitter have normal basal slowing response (positive control). Interestingly, it has been found that *asah-1* knockout mutant worms showed a severely impaired slowing response, indicating that this gene is important for dopamine-driven behaviours even at an early adult stage, and therefore its human orthologue ASAH1 could be implicated in PD. *asp-4* knockouts show a 75.24% average decrease in speed, which is perculiar as it is expected that lysosomal knockouts would hinder this response. More testing would be required to determine the nature that this knockout has on

Human gene	*C. elegans gene*	knockout mutant
GBA	*F11E6.1 (gba-3)*	*F11E6.1(gk3287)*
ATP13A2	*W08D2.5 (catp-6)*	*W08D2.5(ok3473)*
NAGLU	*K09E4.4*	*K09E4.4(gk161202)*
GUSB	*Y105E8B.9*	*Y105E8B.9(ok3031)*
SLC17A5	*C38C10.2 (slc-17.2)*	*slc-17.2(gk821665)*
CTSD	*R12H7.2 (asp-4)*	*asp-4(ok2693)*
ASAH1	*K11D2.2 (asah-1)*	*asah-1(gk738376)*
	F27E5.1 (asah-2)	*F27E5.1(ok564)*
SCARB2	*Y49E10.20 (scav-3)*	*Y49E10.20(ok1286)*
	Y76A2B.6 (scav-2)	*Y76A2B.6(ok877)*
ATP6AP2	*T14B4.3*	-
	R03E1.2 (vha-20)	-
SMPD1	*ZK455.4 (asm-2)*	*asm-2(gk293929)*
	W03G1.7 (asm-3)	*asm-3(ok1744)*
	B0252.2 (asm-1)	*asm-1(gk832037)*
GALC	*C29E4.10*	*C29E4.10(ok2752)*

Table 1. *List of genes for the mutants of which have been investigated for dopaminergic and lysosomal function in this study.*

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other neuronal systems that might cause this response. The *lrk-1* mutant used in this study has increased kinase activity, which mutation in the human LRRK2 orthologue is associated with PD. In this assay, which was peformed in adult, but young worms (day 1 of reproductive maturity) *lrk-1* mutation did not show impaired basals slowing response which is in agreement of previous observations, suggesting an age-dependent loss of dopaminergic function in this genetic PD model. Future experimetns will include the analysis of this behavioural response in aged worms as many risk factors contribute to development of PD in old age.

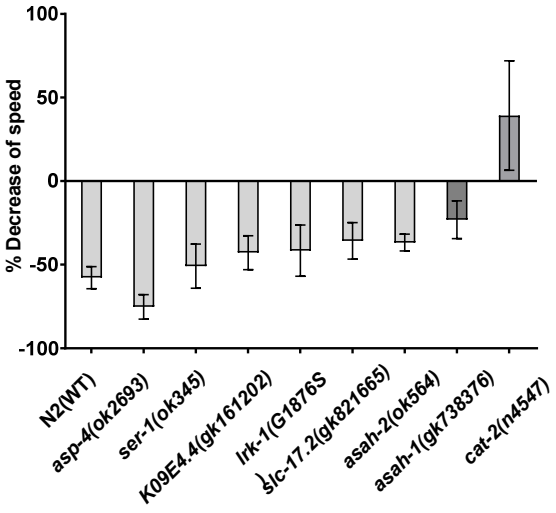

Figure 1. Basal slowing response in the mutant worm strains. *Graphs shows mean change (difference of mean with +SEM) in crawling speed of worms on media without food and with food at the early stage of reproductive adulthood (day 1 adult).*

Western blot analysis of p62 protein levels in the knockout mutant strains have showed increased levels of p62 autophagy receptor protein in most *C. elegans* mutant worms examined (Figure 2), indicating that lysosomal degradation is impaired even in basal conditions upon deletion of these genes. This observation has suggested the the selected lysosomal gene orthologues of PD risk factor candidates indeed are involved in lysosomal clearing mechanisms of *C. elegans*. It has been noted that deletion of these genes caused abnormal growth, with delayed development in most knockout mutant strains analysed, which limited this investigation to the use of then strains shown on Figure 2. The strongest accumulation of p62 is observed in *gba-3*, *catp-6* and *asm-2* knockout mutants. Both GBA and ATP13A2 are established risk factors for PD, while SMPD1 (*asm-2* orthologue) is a novel candidate risk factor

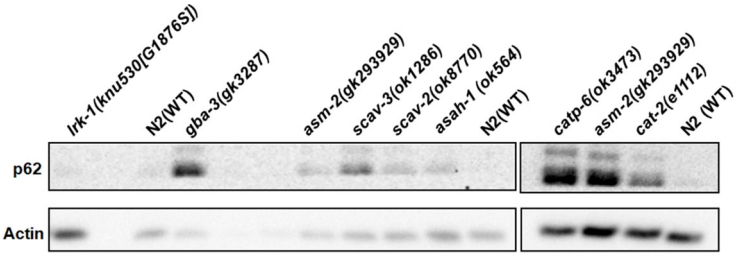

Figure 2. Western blot analysis of p62 protein levels. *p62 protein has been detected in early adulthood (same day as basal slowing assay performed, day 1 adulthood) under basal conditions.*

Future directions:

Due to observed developmental delay and growth defects in multiple mutant worm strains some of the assays have been limited to the use of a subgroup of lysosomal mutants. The planned lysosome visualization by microscopy has not been performed due to the same limitation, as growing and maintenance of these mutant strains took more time learn. Experiments in the host lab is now underway to analyse lysosome structure, distribution and function via fluorescent microscopy. Furthermore, the investigation of the selected lysosomal risk factors for their involvement in age-dependent disease pathology is planned. The next step of this research will involve the visualisation of dopaminergic neurodegeneration upon RNAi depletion of those genes which show defective dopamine-behaviour, including the *asah-1* candidate.

Value of the studentship:

Working in this lab has been one of the most valuable learning experiences in research I have ever received, and I believe it has given me a great advantage on further projects I may choose to undertake. I have learned not only molecular biology techniques, but management and organisational skills with handling my large number of worm strains, large amounts of data analysis and communication skills within a group of scientific peers. I have already begun applying for PhD programmes as a result of the enthusiasm for research these two months have given me. This project will contribute to future projects in the host lab in investigating oligogenic inheritance of PD. Lysosomal genes are in the centre of attention, as deep genome analysis of large PD patient cohorts indicated that Mendelian inherited PD mutations are often associated with mutations in lysosomal genes such as ATP13A2 or GBA. Therefore this study strongly contributed to the development of *C. elegans* models of oligogenic PD inheritance.