

Elucidating the relationship between Complex I dysfunction and autophagy in Parkinson's disease

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Introduction

Parkinson's disease is a neurodegenerative disease which affects approximately 1 in 500 people in the UK (nhs.co.uk). Its characteristic motor symptoms are caused by the loss of dopamine transmission due to cell death in the substantia nigra. Complex I (an enzyme in the mitochondrial respiratory chain) has been found to have decreased activity in Parkinson's disease (Schapira et al, 1990). Dysfunction of autophagy (degradation of organelles such as mitochondria) has also been implicated in Parkinson's (Ebrahimi-Fakhari et al, 2012).

Aims

- To investigate if autophagy is related to dysfunction of complex I (and by extension mitochondrial dysfunction) in Parkinson's disease.
- To investigate how cell density is affected Parkinson's disease.

Methods

Tissue sections came from the Newcastle Brain Tissue Resource. To allow access to the antigens, they were deparaffinised, rehydrated, and subjected to high temperature and pressure in EDTA using an autoclave. Normal goat serum was added to reduce non-specific binding. Then followed incubation with primary antibodies, fluorescent secondary antibodies, a Hoechst stain for nuclei, and Sudan black to counteract autofluorescence. In between each step, a thorough PBS wash was performed to ensure all non-bound antibody/dye was removed. Images were then produced with a fluorescent microscope, as shown in figure 2.

Six autophagy proteins were tested for: P62, ATG5, Beclin 1, LC3B, LAMP, and Parkin. Each section was also tested for C120, a subunit of complex I.

Cresyl fast violet staining was performed on sections from the same patients to allow a cell count.

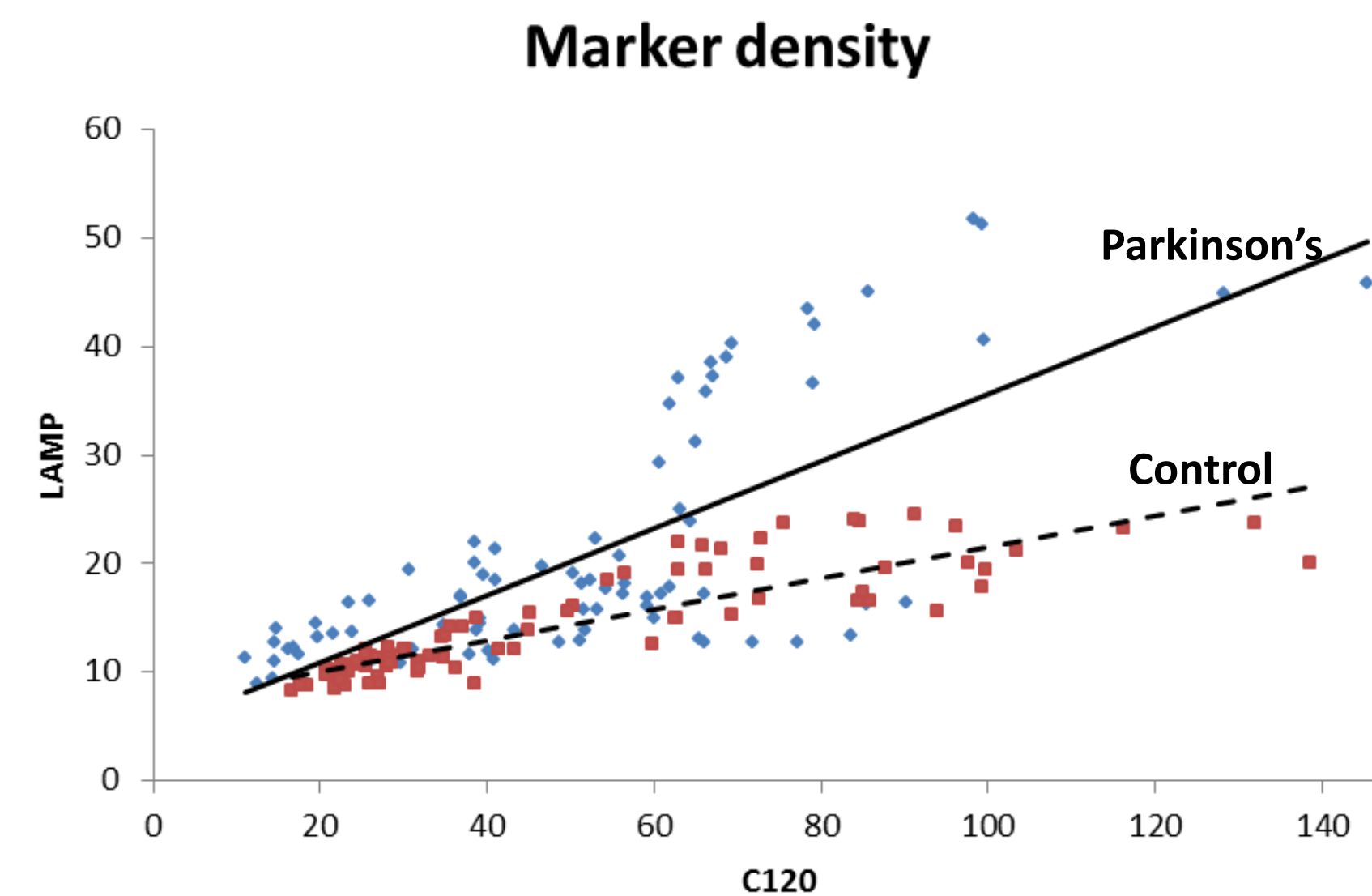


Fig 1. Densitometry values of C120 vs. LAMP2A. LAMP2A (lysosomal-associated membrane protein 2A) is found on the surface of lysosomes. These contain enzymes which degrade mitochondria, therefore they occur at the end of autophagy. This graph shows that in Parkinson's, LAMP accumulates at a higher rate.

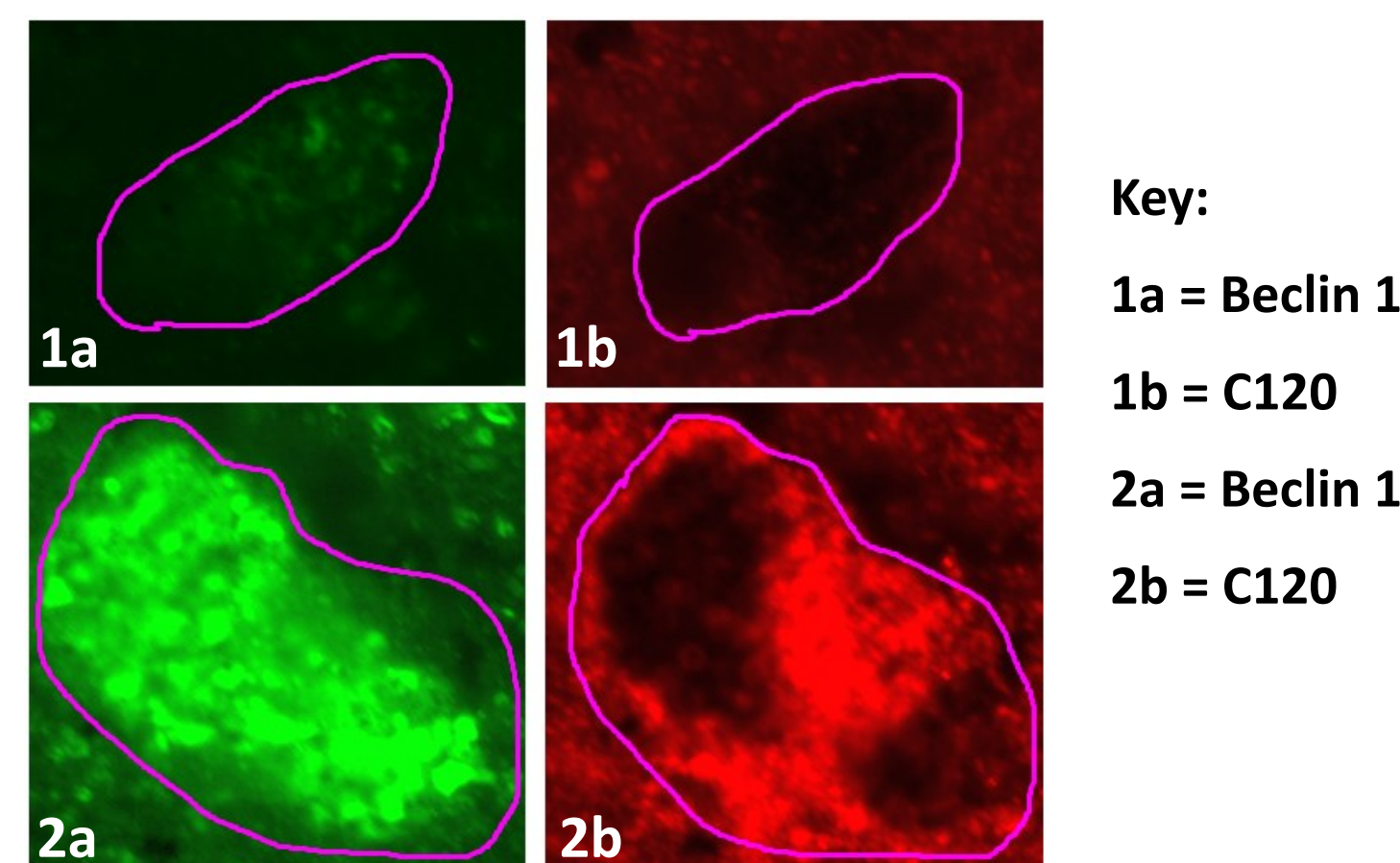


Fig 2. Neuron 1 is deficient in Beclin 1 and C120, neuron 2 is normal for Beclin 1 and C120. Neurons were identified by brightfield and checked for a nucleus using the Hoechst stain. Autofluorescence was accounted for with a no stain control. Intensity was quantified through densitometry software on Axiovision.

Results

All autophagy proteins showed positive correlation with C120. They were elevated for given C120 levels in Parkinson's disease, with the exception of ATG5 and P62. This is shown in figure 1. After statistical transformation, only LAMP was found to have a significantly higher rate of accumulation in Parkinson's compared to controls.

Cell counts from cresyl fast violet stained sections (not shown) found that substantia nigra cell densities were lower in Parkinson's, and that this was statistically significant.

Conclusion

- Autophagy is positively related to mitochondrial function, so one may require the other to function. The greater rate of accumulation of LAMP in Parkinson's indicates accumulation of lysosomes, and therefore autophagy dysfunction.
- Cell density is reduced in Parkinson's disease.

Discussion

Further study would be required to confirm a causative relationship between dysfunction of complex I and autophagy, as this study only shows correlation.

These experiments had a small sample size — 5 Parkinson's patients, 5 controls — so some differences which were not significant in this sample may actually be significant in the whole population.

Acknowledgements

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References

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