Understanding the molecular mechanism of neurodegeneration in mitochondrial disease.

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Introduction

Mitochondria are small, double-membraned organelles present in almost every eukaryotic cell. They function to produce cellular energy, ATP, via a series of five complexes (complex I, II, III, IV and V), which comprise the respiratory chain. Each mitochondrion contains their own DNA, which encodes for components of the mitochondrial respiratory chain. Genetic defects within mitochondrial DNA (mtDNA) affect the production of the complexes in the respiratory chain leading to respiratory deficiency and reduced ATP generation. MtDNA defects are known to result in mitochondrial disease where symptoms may be wide-ranging though neurological impairments are common[1]. Epilepsy affects ~20% of patients with mitochondrial disease[2] and is likely to be a result of abnormal neural activity due to impaired energy production[3]. Respiratory chain deficiency affects multiple cell types in the brain and this project is part of a larger neuropathological series trying to understand the mechanisms underpinning epilepsy in mitochondrial disease. In this study, I focussed on the impact of mitochondrial dysfunction on astrocytes. Astrocytes are glial cells that play an important role in communication between neurons by regulating neurotransmitter metabolism.

Aims

1. To evaluate the density and morphology of astrocytes.
2. Quantify the level of respiratory chain deficiency affecting mitochondria within astrocytes.

Methods

Processing and preparation of 5 µm sections of brain tissue from occipital cortex from:
- **3 disease-control individuals**
- **10 non-disease controls**
- **13 patients with genetically defined mitochondrial disease.**

Immunofluorescence of proteins using the following fluorescently labelled antibodies.
- **Astrocytes (GFAP)**
- **Complex IV (COX-I)**
- **Complex I (CI-20)**
- **Mitochondria (Porin)**

Imaging of sections using a Nikon A1R confocal microscope

Image analysis to get quantitative information regarding the distribution of mitochondria within astrocytes and expression of complexes I and IV.

Results

Increased astrogliosis was detected in patient tissues (Figure 1). A high density of reactive astrocytes were rounded and hypertrophic.

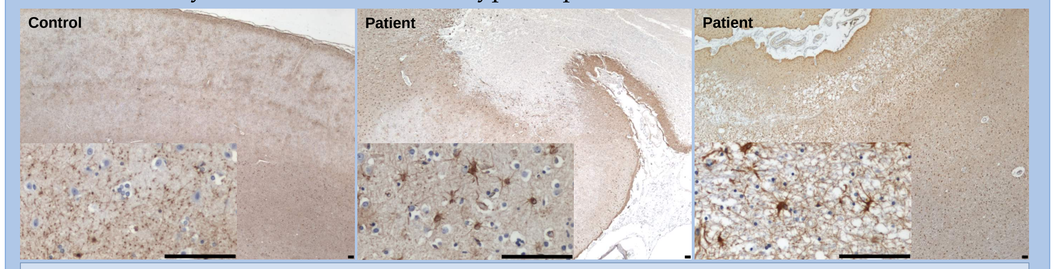


Figure 1 : GFAP staining shows astrogliosis in patients with different genetic mutations causing mitochondrial disease (scale bar = 100 µm).

Patient astrocytes show reduced expression of complex I and IV within mitochondria (Figure 2). This implies that there is a high degree of respiratory chain deficiency in astrocytes.

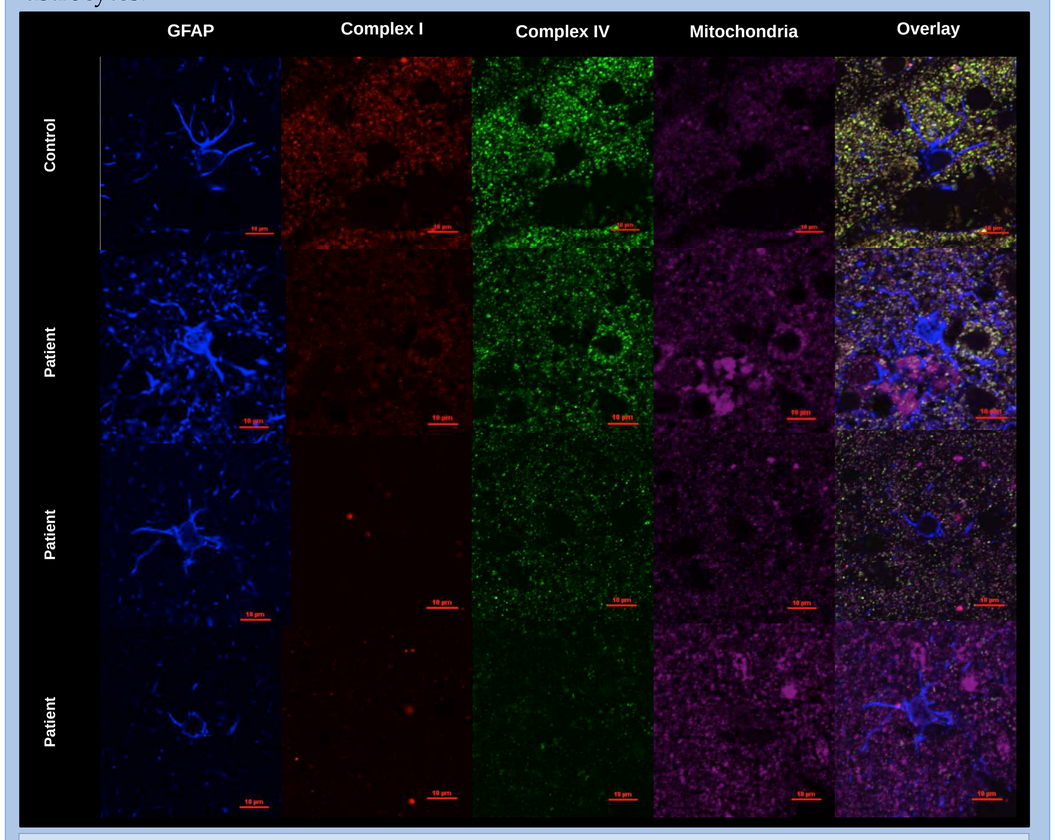
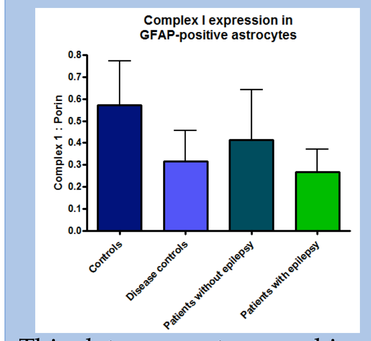
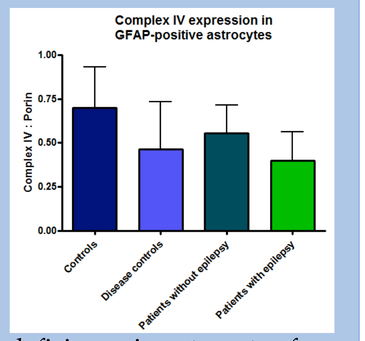


Figure 2: Quadruple immunofluorescence staining reveals that astrocytes (blue) in control tissues show high levels of complex I (red), complex IV (green) and mitochondria (magenta) while reduced expression levels of complex I and IV are observed in patient tissues (scale bar = 10 µm).

Quantification of complex I and IV loss in astrocytes.





This data suggests a combined deficiency in astrocytes from patients with epilepsy. Both complex I and IV are present in a lower ratio in patients with epilepsy than patients without. However, respiratory chain deficiency is also evident in disease controls and some is seen in patients without epilepsy. it is possible that the propagation of seizures is likely to be more complicated.

Conclusions

Astrogliosis is prominent in patients with mitochondrial disease and there is marked complex I and IV deficiency in astrocytes from patients with epilepsy. However, further research will be required to determine how important this is to seizure generation. Indeed work using a functional model would be beneficial to dissect out the potential mechanisms underpinning seizures associated with mitochondrial dysfunction.

Ongoing work

Understanding the impact of respiratory chain deficiency on astrocytic function, particularly on ATP-dependent processes in the synapse. This involves closer examination of glutamate metabolism and regulation of extracellular ions using quantitative immunofluorescence.

References

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