A novel cause of mitochondrial disease: investigating the mechanisms underlying mitochondrial gene expression.

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Introduction

Mitochondria are double membraned organelles present in our cells and function to produce cellular energy using protein complexes of the respiratory chain including Complex I, II, III, IV, V. Mitochondria contain their own DNA (mtDNA) that encodes for proteins and RNAs necessary for energy production. Tight regulation of these genes is required to prevent disease and enable the proper functioning of the mitochondria.

A patient with cardiomyopathy had been biochemically tested and showed a generalised defect of mitochondrial protein synthesis in heart tissues causing a defect in complex I and IV. Exome sequencing identified compound heterozygous mutations in *PTCD1*; to date, mutations in this gene are not a recognised cause of mitochondrial disease. Human pentatricopeptide repeat domain protein 1 (**PTCD1**) is a mitochondrial matrix protein known to be associated with leucine tRNAs, affect the 3'end of mitochondrial tRNA(1) and also believed to be involved in the assembly of respiratory chain complexes(3). PTCD1 is abundant in the testes, skeletal and heart muscle.

My project was to use fixed post mortem samples from heart, skeletal muscle and brain of a 8 month old patient to investigate the extent of the respiratory chain deficiency using immunohistochemistry.

Aim

- To define the extent and pattern of respiratory chain deficiency in patient tissues with PTCD1 mutation.

Method

- Immunohistochemical optimisation of NDUFB8 (recognises 20 kDa of complex I), NDUFS3 (recognises 30 kDa of complex I), SDHA (recognises 70 kDa subunit of complex II), CIIIC2 (recognises core 2 of complex III), COX-I and COX-IV (both recognise subunit I and IV of complex IV, respectively) antibodies was achieved with different antibody dilutions. Primary antibody was detected using a polymer kit, visualised with 3,3'-diaminobenzidine(DAB) which turns brown on reaction with horse radish peroxidase.
- IHC performed on patient and age matched control heart, skeletal muscle and brain sections using the optimised dilutions of the above antibodies.

Results

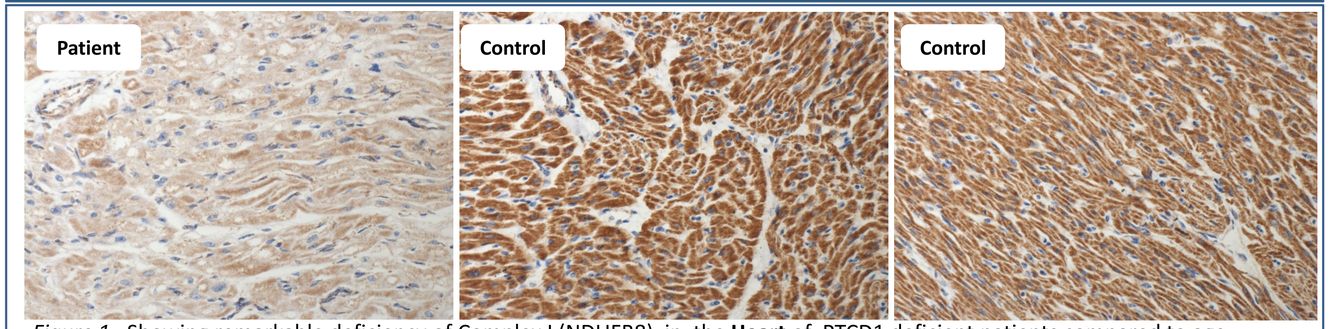

Figure 1 : Showing remarkable deficiency of Complex I (NDUFB8) in the **Heart** of PTCD1 deficient patients compared to age matched controls .

Figure 2 : Showing remarkable deficiency of Complex I (NDUFB8) in the **Skeletal Muscle** of PTCD1 deficient patients compared to age matched controls ..

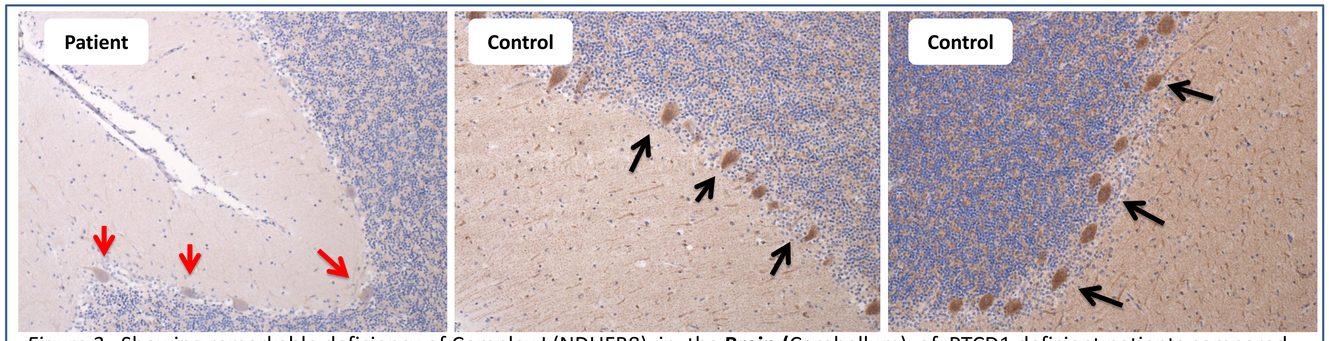

Figure 3 : Showing remarkable deficiency of Complex I (NDUFB8) in the **Brain (**Cerebellum) of PTCD1 deficient patients compared to age matched controls . The arrows points to the neurons with the red arrows highlighting complex I deficiency in patient

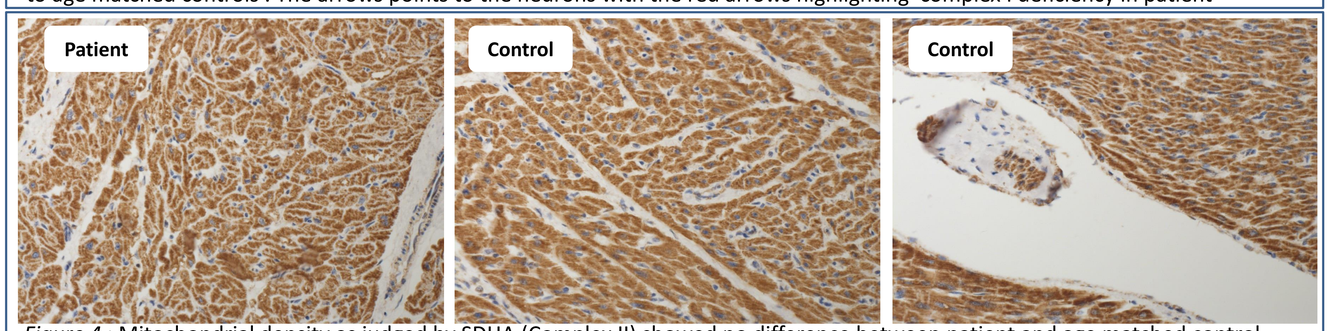

Figure 4 : Mitochondrial density as judged by SDHA (Complex II) showed no difference between patient and age matched control indicating abundant mitochondria In **Heart**.

Conclusion

Upon analysis of the results, remarkable lack of immunoreactivity is observed showing that there is reduced expression of NDUFB8, NDUFS3 which are components of the complex I and COX-I and COX-IV which are components of complex IV of the electron transport chain in the heart , skeletal muscle and brain sections which indicates that the deficiency in complex I and IV is not regional but cuts across various organs of the body.

However, there is no difference in the expression of CIIIC2 and SDHA (which was used as a mitochondrial mass marker as it is nuclear encoded) showing that these complexes are not affected in all the sections – heart, skeletal muscle and brain.

This study show that *PTCD1* mutation is responsible for the deficiencies of the respiratory chain complexes in tissues and therefore the inability to generate the appropriate cellular energy needed.

Ongoing Work

- Analyse the results of the immunohistochemistry performed on the basal ganglia and parietal lobe of the brain.
- IHC measure PTCD1 protein levels in tissue using an appropriate PTCD1 antibody.
- Carryout confirmatory tests to show that the patient lacked no other mutations in the mtDNA.

References

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