Investigating the Molecular Basis of Mitochondrial Disease: A Novel UQCRH Mutation causes a Defect in Complex III Assembly and Activity

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Introduction

- Mitochondria produce ATP (adenosine triphosphate) which is the cell's primary energy currency.
- Mitochondrial disease occurs when these ‘powerhouses’ are dysfunctional and cells produce insufficient ATP.
- ATP is a product of oxidative phosphorylation (OXPHOS), a process requiring five protein complexes (I-V).
- Mutations within either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) impair OXPHOS.
- Complex III (CIII or cytochrome c oxidoreductase) contains 11 mtDNA and 10 nDNA-encoded subunits.
- CI mutations are diagnostically difficult to identify and are 890.

**Experimental Methods**

- Next Generation Whole Exome Sequencing (WES) identified a homozygous UQCRH deletion mutation.
- OXPHOS proteins from primary patient fibroblasts were analysed.
- Antibodies specific to different OXPHOS complex subunits were used to visualise the relevant proteins.

**Results**

- Western Blot (Figure 5A) detected decreased levels of complex III in the patient compared to controls.
- Blue Native PAGE showed a CIII band of a lower molecular weight protein complex for the affected patient.
- A different UQCRH antibody will be used in future Blue Native PAGE to obtain clearer bands when imaged and Sanger sequencing confirming the deletion breakpoint.

**Discussion and Conclusion**

- Decreased expression of Complex III in the patient presenting with mitochondrial disease:
  - The Western Blot (Figure 5A) detected decreased levels of CIII subunit UQCR2 in the patient fibroblasts when compared to the controls. Reduced steady-state levels of CIII negatively affects the availability of CIII in the OXPHOS process and the output of ATP.
  - Assembly of Complex III is incomplete in the affected patient mitochondria.
  - In the Blue Native (Figure 5B), the patient CIII band has a reduced molecular weight (MW) because it has travelled lower down than the controls.

**Future Work**

- A different UQCRH antibody will be used in future Blue Native PAGEs to obtain clearer bands when imaged and to determine if the UQCRH subunit is truly absent.
- Patient fibroblast cells will be subject to lentiviral transduction with wild-type cDNA. This is where DNA without the patient’s biochemical defects is introduced into patient cells to effect rescue and confirm the pathogenic nature of the mutation.
- Proteomic studies for patient CIII will help determine what protein segment is absent.

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**References**