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

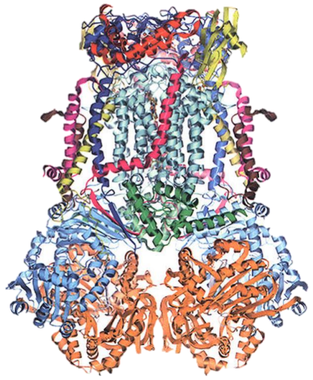
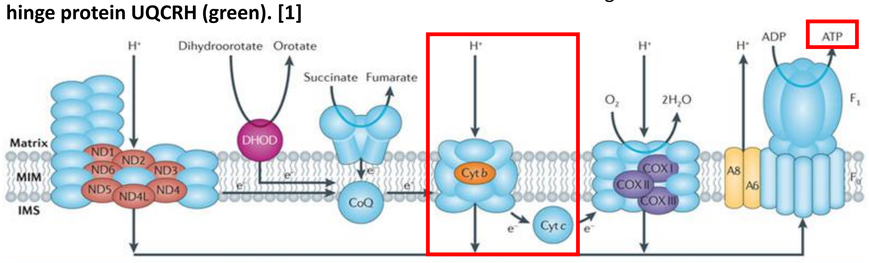


Figure 1: Complex III (Cytochrome c oxidoreductase) Structure. 11 subunits include hinge protein UQCRH (green). [1]

- 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 1 mt-DNA and 10 nDNA-encoded subunits.
- CIII mutations are diagnostically difficult to identify and are therefore viewed as the rarest of the OXPHOS defects [2].
- The centre of CIII contains a structural hinge protein called UQCRH (nDNA-encoded).
- An understanding of how individual patient nDNA mutations affect assembly and performance of the OXPHOS complexes is crucial to further define the causes and molecular mechanisms of these debilitating diseases.



Polypeptides	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded subunits	7	0	1	3	2
nDNA-encoded subunits	~39	4	10	10	~14

Figure 2: Graphic of the functional components of OXPHOS. Complex subunits are encoded by nDNA (blue) or mtDNA (coloured). ATP is produced as an end-product from complex V. The ATP and complex III are highlighted in red boxes. [3]

Patient Profile

The proband (arrowed) is an 11-year-old child of first-cousin parentage who presents with recurrent episodes of severe ketoacidosis, excess blood ammonia and hypoglycaemia - leading to episodes of encephalopathy, although brain MRI is normal. A first cousin, now 8 years of age, presented with a very similar clinical course requiring admission to PICU, prompting genetic studies.

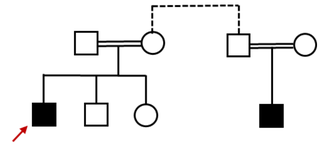


Figure 3: Pedigree Chart of Patient

Aims

1. To assess the functional effects of a candidate *UQCRH* variant by measuring the steady-state levels, subunit assembly and enzyme activity of OXPHOS complexes
2. To provide the family with a genetic diagnosis and molecular basis of the disease for the purpose of informing future reproductive choices

Experimental Methods

- Next Generation Whole Exome Sequencing (WES) identified a homozygous *UQCRH* deletion mutation
- OXPHOS proteins from primary patient fibroblasts were analysed
- The protein analytical techniques of Western Blot and Blue Native PAGE helped to determine protein steady-state levels and subunit assembly respectively
- Antibodies specific to different OXPHOS complex subunits were used to visualise the relevant proteins

Results

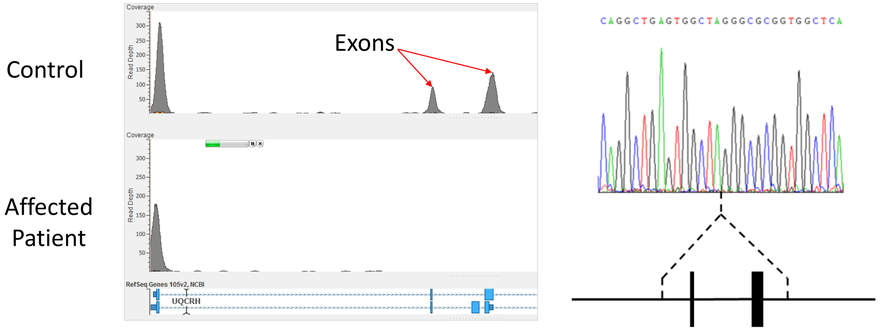


Figure 4: WES data showing the proband patient lacks coverage for 2 exons compared with the control (left) and Sanger sequencing confirming the deletion breakpoint (right). Both parents were heterozygous.

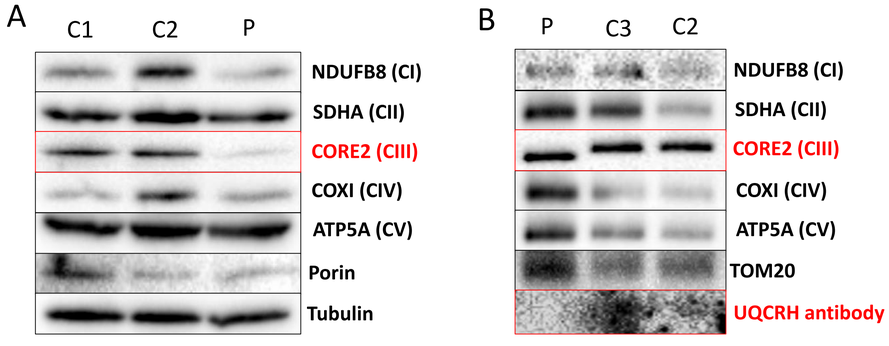


Figure 5: Western Blot showing a decrease in CIII subunits in the patient compared to controls (left). Blue Native PAGE (right) showing a CIII band of a lower molecular weight protein complex for the affected patient compared to the controls. Clarity of the CIII UQCRH subunit bands is poor (right) but potentially shows there is no band for the patient mitochondria. Porin, TOM20 and tubulin were used as loading controls.

Figure 6 (left): Enzyme Activities of OXPHOS Complexes I-IV. Patient enzymes (blue) have lower activities for CI and CIII than the controls (red).

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 UQCRC2 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
  - The reduction in MW is small so an absent or shortened protein subunit may explain this
  - An assumption would be that *UQCRH* (hinge protein) is absent since its gene sequence is mutated. This would be consistent with the observed size change of assembled CIII, but requires further confirmation
- **Complex I and III have reduced enzyme activity in the patient**
  - Reduced CI and CIII enzyme activities (Figure 6) contribute to mitochondrial disease presentation as OXPHOS and its ATP production are less active overall

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

References

1. Yu CA et al. Bioenerg Biomembr. 1999; 31(3): 191-9
2. Fernández-Vizarra E. et al. Front. Genet. 2015; 6:134.
3. Schon EA et al. Nat. Rev. Genet. 2012; 13: 878-890.

Acknowledgements

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