The molecular genetic basis of mitochondrial complex I deficiency

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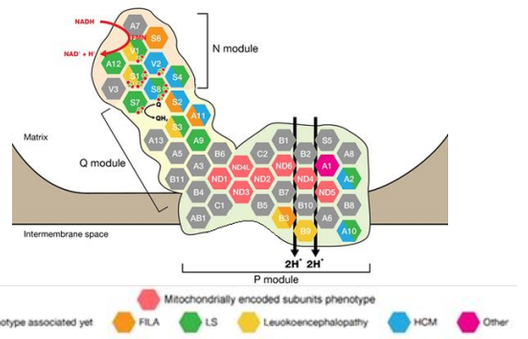
Introduction

Mitochondrial disease is a debilitating illness estimated to affect 1 in 5000 people in the UK [1]. Of these, isolated complex I (NADH:ubiquinone oxidoreductase) deficiency is one of the most common, often presenting at birth or in early childhood. Symptoms are varied and can include elevated blood lactate, cardiomyopathy, hepatopathy and changes on brain MRI.

Complex I is the first enzyme in the mitochondrial respiratory chain and reoxidises NADH generated by cellular metabolism. The complex is under dual genetic control – made up of both mitochondrial DNA (mtDNA) and nuclear-encoded subunits – as well as numerous assembly factors key to ensuring normal function. Mutations in any of these elements can cause reduced ATP production and the clinical and genetic heterogeneity leads to considerable diagnostic challenges for clinicians.

In the absence of effective treatments for mitochondrial disease, providing reproductive counselling for patients with inherited mitochondrial disease is crucial. The current molecular genetic testing strategy screens the mitochondrial genome and 10 of the 50 nuclear genes linked to complex I deficiency, thereby only obtaining a conclusive genetic diagnosis in ~50% of patients[2]. There is a need to improve this detection rate by introducing new technologies that can rapidly screen all known genes implicated (Figure 1).

Figure 1. Schematic showing the organisation of the 44 structural subunits in assembled complex I, the subunits are coloured according to their associated clinical phenotypes. Adapted from [3]



Aims

1. To define the molecular basis of isolated respiratory chain complex I deficiency in a small cohort of paediatric patients using next generation sequencing.
2. To improve and to expedite the diagnosis of mitochondrial diseases using Next Generation Sequencing (NGS) for paediatric patients with isolated Complex I deficiency.

Patients and Methods

- Patient 1 was an infant presenting with Leigh syndrome and an isolated complex I deficiency in muscle (40% of control activity). This disease is characterized by psychomotor regression which in the patient manifested with vomiting, diarrhoea and difficulty swallowing, resulting in eating problems and failure to thrive. Patient 1 died at age 4 months.
- Patient 2 presented at age 2 months with hypertrophic cardiomyopathy and lactic acidosis (20mmol/L). Her muscle biopsy revealed an isolated complex I deficiency (30% of controls). She died aged 3 months.
- An Ampliseq panel selective for the 50 genes relevant in complex I deficiency was designed
- Massively parallel sequencing was carried out using the IonTorrent PGM on stored patient DNA
- A plugin generates a file containing all variants. Missense variants were annotated using prediction software to investigate their potential impact
- Clinically relevant variants which had a putative deleterious effect were then confirmed by PCR and Sanger sequencing
- The resultant data was analysed using Mutation Surveyor v3.24 according to Diagnostic protocols enabling any positive results to be conveyed to the referring clinicians

Results

Patient 1

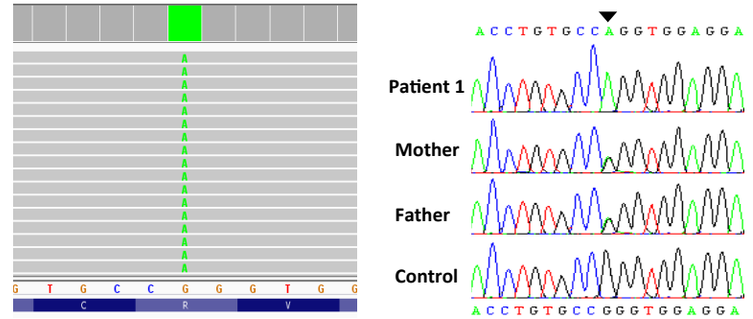


Figure 2: NGS trace (left) showing a previously reported pathogenic, homozygous *NDUFS2* mutation at c.998G>A, p.(Arg333Gln) [3], confirmed by Sanger sequencing (right)

Patient 2

Figure 3: NGS trace (left) showing a novel, heterozygous *ACAD9* mutation at c.1552C>T, p.(Arg518Cys), confirmed by Sanger sequencing (right)

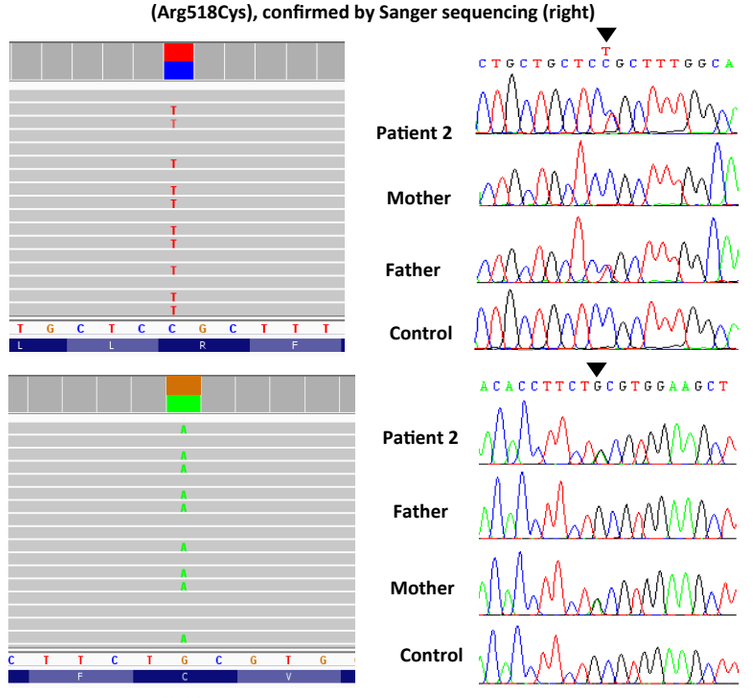


Figure 4: NGS trace (left) showing a novel heterozygous *ACAD9* mutation at c.1715G>A, p.(Cys572Tyr), confirmed by Sanger sequencing (right)

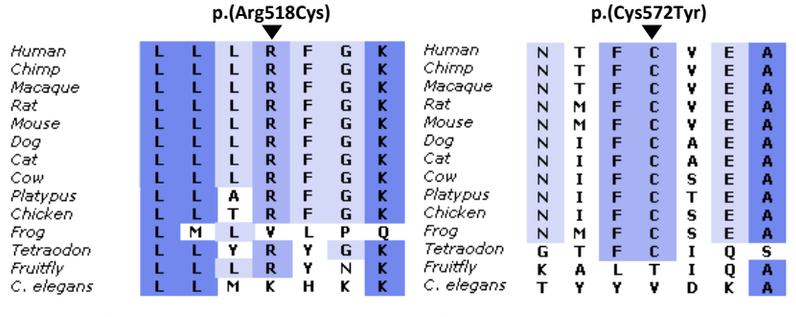


Figure 5: Amino acid conservation of Patient 2's novel *ACAD9* mutations.

Discussion

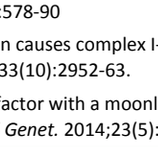
Present diagnostic procedures for mitochondrial disease in paediatric patients involve an invasive muscle biopsy under general anaesthetic to obtain samples of affected tissue. Samples are then processed by Sanger sequencing, which takes about 12 weeks to analyse mtDNA and the 10 genes encoding structural subunits.

The Ampliseq and IonTorrent PGM testing strategy provides a more rapid method for obtaining a genetic diagnosis by working with blood samples from patients – a muscle biopsy may not be required and results are available within a week.

This technique is able to analyse all 50 nuclear-encoded candidate disease genes of complex I deficiency. It is also more cost-effective.

The pathogenic c.998G>A, p.(Arg333Gln) *NDUFS2* mutation harboured by Patient 1 affects a structural subunit of Complex I and causes Leigh Syndrome. This mutation was homozygous and It is important to note that this homozygous mutation was detected within a consanguineous family (Figure 6).

Figure 6. Pedigree of Patient 1
Parents are first cousins.
Patient 1 is their first daughter.



Mutations in *ACAD9* are a well-recognised cause of isolated complex I deficiency and cardiomyopathy [5]. The novel c.1552C>T, p.Arg518Cys and c.1715G>A, p.(Cys572Tyr) *ACAD9* variants identified in Patient 2 are excellent candidate mutations. They are thought to cause a change in function of a complex I assembly factor, culminating in hypertrophic cardiomyopathy. The pathogenicity of these novel heterozygous *ACAD9* mutations warrants further investigation.

Conclusions

1. This strategy has established a genetic diagnosis for two paediatric patients with isolated complex I deficiency.
2. The speed and potential detection yield for paediatric mitochondrial disease patients enables NGS to be a feasible diagnostic tool.
3. NGS analysis could eventually remove the need for muscle biopsy from the diagnostic testing pipeline for those patients with a clear mitochondrial phenotype. Muscle biopsy is an expensive, invasive and high-risk procedure.
4. Establishing a genetic diagnosis enables timely reproductive counselling and ultimately prevent the transmission of serious mitochondrial disease.

References

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