Lay Summary

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Developing novel diagnostic and functional mitochondrial assays in muscle and skin biopsies using combinatorial immunohistochemical, in situ hybridisation and proteomic-based techniques

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Mitochondria are small parts of mammalian cells that generate energy. Each mitochondrion contains many copies of mitochondrial DNA (mtDNA), the genetic code for making proteins needed for energy production. Mitochondrial diseases are rare inherited metabolic disorders that can present at any age. Tissues with high energy requirements such as the brain, heart and skeletal muscles are frequently affected. The clinical diagnosis can be very challenging, and establishing a genetic diagnosis can be a lengthy, complicated process for many patients. Most individuals with suspected mitochondrial disease will have further laboratory tests, including skeletal muscle and skin biopsies taken for confirmation of the clinical diagnosis. By combining new techniques in tissue staining and microscopy, we have developed sophisticated methods that can simultaneously pinpoint the quantity of mtDNA and the amount of mitochondrial proteins essential in mitochondrial energy production in single muscle fibres. We can now measure levels in 100s-1000s of fibres in thin muscle slices taken from diagnostic biopsies. We are developing digital techniques to analyse the complex data using powerful digital software. We will also use a sophisticated technique called single-cell RNA sequencing, that allows us to study the RNA 'message' for the entire cell machinery that maintains and recycles damaged mitochondria. This technique can inform us which genes are 'switched on' and switched off' in biopsies of individuals with mitochondrial disease compared to unaffected controls. We can investigate these potential links further in patient skin cells growing in the laboratory ("modelling" mitochondrial mutations in fibroblasts). We expect these approaches to improve our ability to detect mitochondrial protein and DNA defects in single cells, and potentially identify new markers for diagnosing mitochondrial disease and monitoring disease activity. In the long term, better diagnosis and understanding will lead to new treatments for these devastating diseases.