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Challenges in Diagnosis of Mitochondrial Respiratory Chain Complexes Disorder in Human Skin Fibroblasts

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Abstract

Mitochondrial respiratory chain (MRC) disease is a heterogeneous group of disorders characterized by impaired energy metabolism due to genetically-based oxidative phosphorylation (OXPHOS) dysfunction. The diagnosis is challenging as the preliminary diagnosis is solely based on the clinical basis. The present study describes the biochemical and molecular approaches used to confirm MRC disease. Skin biopsies were taken from 24 infants suspected of having inborn errors of metabolism and further being processed to acquire fibroblasts for all experimental work. Biochemical approaches in MRC diagnosis involved kinetic enzymatic assay and Western-Blot analysis of MRC proteins; namely Complex 1 to Complex V. The kinetic activity of each enzyme complex was captured using microplate spectrophotometer at a specific wavelength. The enzyme activities were expressed as a rate (nmol/min) per mg of protein. In Western-blot analysis, proteins were separated by electrophoresis and electro-blotted onto PVDF transfer membrane, followed by immuno-binding reaction of antibody against targeted proteins subunit. The protein immune-blots were visualised and estimated through a Protein Image Analyser. In any case of abnormality detected by both analyses, the mitochondria DNA sequencing would be performed to detect for any mutation. Out of 24 patients, 4 patients were found to have MRC complex I and IV deficiencies by enzymatic assay, with a moderately low protein detection through Western blot analysis. 2 out of these 4 abnormal patients were preliminarily diagnosed with fatty acid oxidation deficiencies, suggesting a secondary to OXPHOS defects. Through DNA sequencing, one patient was concluded for a heterozygous mutation for SURF-1 (gene encodes an assembly factor of mitochondrial complex IV), therefore the patient is a carrier. Despite the success in getting a conclusive and reliable diagnosis, the study also recorded few important challenges during the process, which includes difficulties in obtaining skin biopsy samples from the patients, the requirement for a large number of cells and fresh samples, and also the preferences of using isolated intact-mitochondrial for enzymatic assay analysis.

Keywords: Mitochondrial respiratory chain; skin biopsy; fibroblasts

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