

## DIAGNOSIS

# The Promise of DNA Analysis in Understanding Mitochondrial Disease

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As researchers chart the human genome, DNA analysis is providing new insights into the genetic bases of many diseases. Analytic methods that have been used to uncover nuclear DNA mutations are now being applied with some success to the mitochondrial genome. Unfortunately, the most common method of searching for new DNA mutations, gel electrophoresis, is time- and labor- intensive, and therefore very expensive.

A new process, referred to as DNA chip technology, offers the promise of automation. In this technique, large groups, or arrays, of different, short wild-type genetic sequences are placed on a solid support, or chip. These arrays are then chemically joined, or hybridized, with samples of DNA from patients and their family members after PCR amplification. The presence of mutations causes incomplete hybridization, which shows up as variations in fluorescence that can then be rapidly analyzed by a scanner.

DNA chip technology is evolving rapidly. These miniature chips, once perfected, will allow researchers to rapidly compare mutant and wild-type genetic sequences, much as a computer can compare and analyze two sets of data, and report on the variances. Arrays can be tagged by fluorescence for multicolor detection. Enzymes are used to facilitate identification of mutations, acting as tagged targets for comparison.

This technique harbors enormous potential for looking at the variation inherent in mitochondrial disease. For example, in the 1996 genome issue of *Science*, Chee et al, (Accessing Genetic Information with High-Density DNA Arrays. 1996: 274, 610-614), reported on a tiling array for the entire mitochondrial genome. Chee et al were able to image hybridization patterns of long PCR products of the entire

mitochondrial genome in less than 10 minutes, identifying 99% of the mitochondrial sequence correctly. Three disease-causing mutations from a patient with Leber hereditary optic neuropathy were identified correctly, and a total of seven errors and new polymorphisms from previously unsequenced regions were identified. The analysis of ten genomes unambiguously identified 505 polymorphisms between the individuals studied. Equally important, no false positives were detected in the sequence analysis.

At present, the best understood of the mitochondrial abnormalities are the maternally derived mitochondrial DNA

(mtDNA) mutations. Once researchers are able to characterize these abnormalities using DNA chip testing, we will be able to screen large populations for variations in specific phenotypes, and learn more about clinical conditions which, as of yet, are poorly defined and highly variable, but still suspected to be influenced by mtDNA variations. In addition, low-cost rapid screening may allow fundamental research studies of mitochondrial variation within an individual, allowing a better understanding of the process of intra- and intercellular heteroplasmy, and further elucidating the role of mitochondrial variation in health and disease.

## A New Horizon for the Diagnosis of Mitochondriopathies

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The evaluation of a person with a suspected mitochondrial disorder is currently difficult, frequently requiring a muscle biopsy for confirmation, along with DNA testing of several tissues to detect mutations. A noninvasive means to identify those patients who truly have disorders of mitochondrial function would greatly assist some patients by eliminating unnecessary testing. One possible way to achieve this is magnetic resonance imaging with specific substrate detection (lactate and pyruvate). This technique has not yet been widely used.

Another possibility is the measurement of oxygen consumption using tissue oximetry, recently described by Abe, et al, from Osaka, Japan in *Neurology* (Measurement of tissue oxygen consumption in patients with mitochondrial myopathy by noninvasive tissue oximetry. 1997: 49(3), 837-841). In this technique, a transcutaneous oxygen-sensing electrode was attached to the quadriceps muscles of adults with known mitochondrial defects in order to measure oxygen consumption during exercise. All four patients studied showed impaired oxygen consumption compared to normal controls. Further refinements of this technique to allow it to be applied to children who cannot cooperate by exercising on request could markedly improve the clinician's ability to determine which patients need further work-up.