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Scientists create one-step gene test for mitochondrial diseases

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More powerful gene-sequencing tools have increasingly been uncovering disease secrets in DNA within the cell nucleus. Now a research team is expanding those rapid next-generation sequencing tests to analyze a separate source of DNA—within the genes inside mitochondria, cellular power plants that, when abnormal, contribute to complex, multisystem diseases.

The study team, headed by a specialist in mitochondrial medicine at The Children's Hospital of Philadelphia (CHOP), adapted next-generation sequencing to simultaneously analyze the whole exome (all the protein-coding DNA) of nuclear genes and the mitochondrial genome. "A first step in developing treatments for a disease is to understand its precise cause," said Marni J. Falk, M.D., the director and attending physician in the Mitochondrial-Genetic Disease Clinic at Children's Hospital. "We have developed a one-step, off-the-shelf tool that analyzes both nuclear and mitochondrial DNA to help evaluate the genetic cause of suspected mitochondrial disease."

Falk and colleagues describe their customized, comprehensive test, which they call the "1:1000 Mito-Plus Whole-Exome" kit, in the journal *Discovery Medicine*, published Dec. 26, 2012. Her co-corresponding author, biostatistician Xiaowu Gai, Ph.D., now of the Loyola University Stritch School of Medicine, collaborated on developing the test while at Children's Hospital.

While each mitochondrial disease is very rare in the population,

hundreds of causes of mitochondrial diseases are known. Some originate in mutations in DNA specific to the mitochondria, tiny structures located outside the cell nucleus, while many other mitochondrial diseases are based in nuclear DNA genes that affect mitochondrial function. The role of mitochondria in human disease has been recognized only since the 1980s, based on pioneering research by Douglas C. Wallace, Ph.D., now at Children's Hospital, and a co-author of the current study.

Many mitochondrial diseases remain poorly understood. One complicating factor is heteroplasmy—a mixture of mutated and normal mitochondrial genomes within the same cells or tissues. In contrast to conventional gene sequencing, which can detect only heteroplasmic mutations that reach levels of at least 30 to 50 percent, the customized kit has the sensitivity to detect mitochondrial genome mutations present at levels as low as 8 percent. To achieve their results, the study team adapted an existing whole-exome sequencing kit from Agilent Technologies, expanding it to encompass the mitochondrial genome.

The availability of the new kit, said Falk, if used for either clinical or research purposes, may shorten the "diagnostic odyssey" experienced by many patients and families seeking the cause of debilitating and puzzling symptoms. "Many families travel from one specialist to another for years, searching for the cause of their rare disease," she says. Specific treatments are not always available, but identifying their disease cause may be the first step toward discovering treatments.

A second recent study by Falk and colleagues reviews progress in diagnosing mitochondrial disease, through their experience at a single center over a rapidly changing three-year period before whole-exome sequencing was generally available. The retrospective review in *Neurotherapeutics*, published Dec. 27, 2012, covers 152 child and adult patients evaluated at CHOP's Mitochondrial-Genetics Diagnostic Clinic from 2008 to 2011.

"Before 2005, very few individuals could receive definitive molecular diagnoses for mitochondrial diseases, because of limitations in both knowledge and technology," said Falk. "Since that time, the clinical ability to sequence whole mitochondrial DNA genomes has significantly improved the diagnosis of many mitochondrial disorders."

During the study period covered in the review article, the clinic at CHOP confirmed definite mitochondrial disease in 16 percent of patients and excluded primary mitochondrial disease in 9 percent. While many diagnostic challenges clearly remain, Falk says the advent of massively parallel nuclear exome sequencing is revealing increasingly more of the genes in nuclear DNA that affect mitochondrial function, and the precise genetic disorder in a given patient, even if it is novel or uncommon. She added that molecular genetics is yielding a more nuanced understanding of the cellular pathways underlying symptoms in many mitochondrial disorders. "Those pathways offer potential new targets for treating these disorders," said Falk.

More information: "Mitochondrial Disease Genetic Diagnostics: Optimized Whole-Exome Analysis for All MitoCarta Nuclear Genes and the Mitochondrial Genome," *Discovery Medicine*, published online Dec. 26, 2012. bit.ly/Vyb4lc.

Provided by Children's Hospital of Philadelphia