A team led by The Children's Hospital of Philadelphia's Marni J. Falk, M.D., has expanded next-generation gene sequencing tools designed to unlock the secrets hidden in nuclear DNA to analyze a separate source of DNA — that found within mitochondria. Mitochondria, which contain their own DNA, are key suppliers of the energy needed for the multiple functions of our cells, and play a pivotal role in human health and disease.

“A first step in developing treatments for a disease is to understand its precise cause,” said Dr. Falk, director of 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.”

The investigators described their customized, comprehensive test — which they call the “1:1000 Mito-Plus Whole-Exome” kit — recently in Discovery Medicine. To achieve their results, the study team adapted an existing whole-exome sequencing kit from Agilent Technologies, expanding it to encompass the mitochondrial genome.

While individual mitochondrial diseases are very rare, hundreds of causes of mitochondrial diseases are known. Some originate in mutations in DNA specific to the mitochondria, 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, and is largely based on the pioneering research by CHOP’s Douglas C. Wallace, Ph.D., a co-author of the current study. However, 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, Dr. Falk’s kit has the sensitivity to detect mitochondrial genome mutations present at levels as low as 8 percent.

The availability of the new test may shorten the “diagnostic odyssey” experienced by many patients and families seeking the cause of debilitating and puzzling symptoms, said Dr. Falk. “Many families travel from one specialist to another for years, searching for the cause of their rare disease.”

Specific treatments are not always available, but identifying their disease cause may be the first step toward discovering treatments, Dr. Falk noted.

A second study led by Dr. Falk reviews progress made in diagnosing mitochondrial disease at a single center over a rapidly changing three-year period before whole-exome sequencing was generally available. The retrospective review, published recently in Neurotherapeutics, 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 Dr. 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, 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, 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, Dr. Falk said. Molecular genetics is yielding a more nuanced understanding of the cellular pathways underlying symptoms in many mitochondrial disorders, she added.

“Those pathways offer potential new targets for treating these disorders,” said Dr. Falk.
An extensive genomic study of the childhood cancer neuroblastoma reinforces the challenges of treating the most aggressive forms of this disease. Contrary to expectations, the researchers found relatively few recurrent gene mutations — mutations that would suggest new targets for neuroblastoma treatment. Instead, the investigators have refocused on how neuroblastoma tumors evolve in response to medicine and other factors.

“This research underscores the fact that tumor cells often change rapidly over time, so more effective treatments for this aggressive cancer will need to account for the dynamic nature of neuroblastoma,” said the study’s leader John M. Maris, M.D., director of The Children’s Hospital of Philadelphia’s Center for Childhood Cancer Research.

Striking the peripheral nervous system, neuroblastoma usually appears as a solid tumor in a child’s chest or abdomen. Though only comprising 7 percent of all childhood cancers, it causes 10 to 15 percent of all childhood cancer-related deaths. Neuroblastoma is also notoriously complex, with a broad number of gene changes that can give rise to the disease.

Dr. Maris led a multicenter research project, as part of the National Cancer Institute’s TARGET (Therapeutically Applicable Research to Generate Effective Treatments) initiative, which published its findings recently in Nature Genetics. In addition to Dr. Maris, contributors to the study included the Broad Institute’s Matthew Meyerson, M.D., Ph.D., and Marco A. Marra, Ph.D., of the University of British Columbia.

The largest genomic study of a childhood cancer to date, the TARGET project analyzed DNA from 240 children with high-risk neuroblastomas. Using a combination of whole-exome, whole-genome, and transcriptome sequencing, the study compared DNA from tumors with DNA in normal cells from the same patients, with the goal of mapping out a limited number of treatment strategies. This approach would have represented a significant step forward in the personalization of neuroblastoma therapy.

Along these lines, researchers at Children’s Hospital and other centers had previously discovered neuroblastoma-causing mutations, such as those in the ALK gene. In the subset of patients carrying this mutation, oncologists can provide effective treatments tailored to their genetic profile.

“A few years ago, we thought we would be able to sequence the genomes of individual patients with neuroblastoma, detect their specific cancer-causing mutations, and then select from a menu of treatments,” said Dr. Maris.

However, though the TARGET researchers confirmed that roughly 10 percent of the patients studied had ALK mutations, while also finding a handful of other gene mutations with percentages in the single digits, there were relatively few recurrent mutations in somatic (non-germline) cells. “The relative paucity of recurrent mutations challenges the concept that druggable targets can be defined in each patient by DNA sequencing alone,” noted the study’s authors.

In the absence of frequently altered oncogenes that drive high-risk neuroblastomas, the investigators concluded that some cases may result from other changes, such as rare germline mutations or epigenetic modifications during tumor evolution. Going forward, researchers may need to turn to functional genomics, to learn which tumors will or won’t respond to treatments while also going beyond a static picture of a cancer cell with fixed genetic contents.

Future strategies could involve devising interventions to deal with dynamic tumor cells that evolve during nervous system development, Dr. Maris noted.

“Personalized medicine is more complex than we had hoped,” said Dr. Maris. “While there are successes such as those in treating patients whose tumors harbor ALK mutations, this study implies that we must think very differently about how we’ll use genomics to define treatment.”
A new study verifies the long-controversial belief that a few children, in exceptional cases, can “recover” from autism. The study, which included investigators from the Center for Autism Research, is the first solid science to confirm that, however rarely, with the help of behavioral therapy some children can make such great improvements that they no longer qualify as having autism.

Published online Wednesday in the *Journal of Child Psychology and Psychiatry*, the study was led by the University of Connecticut’s Deborah Fein, Ph.D. Investigators from Queens University in Ontario, Canada, the Institute of Living in Hartford, Conn., and New York, N.Y.’s Child Mind Institute also contributed to the study. Director of the Center for Autism Research Robert Schultz, Ph.D., served as one of the study’s co-authors.

The researchers sought to explore whether the autism-recovery phenomenon was the result of an initial misdiagnosis, or whether the children’s recovery was complete. For example, the investigators asked, could the recovered children have retained significant social impairments despite losing the technical diagnosis of autism?

To test and document children who were diagnosed with autism at a young age but who no longer were qualified as such, Dr. Fein and her group compared three groups of patients. They examined a group with a history of autism and “optimal outcomes,” a second group who had high-functioning autism, and a group of children who were developing typically.

The study’s results “clearly demonstrate” that some children who had previously been diagnosed with autism later recovered, to the point that their communication and social skills were comparable to those of typically developing children.

“This study is the first to establish with firm evidence that a small percentage children with an autism spectrum disorder in early childhood can be essentially symptom free by adolescence,” said the Center for Autism Research’s Dr. Schultz. “Although there has been ample anecdotal evidence to this effect for many years, it was never clear that those cases were accurately diagnosed in the first place. This study puts that concern to rest and raises hope for families that some children do show dramatic recovery and what we are calling an “optimal outcome”.

As might be expected, Dr. Feinstein’s study has already garnered a fair amount of media attention, from both the *New York Times* and the autism advocacy organization *Autism Speaks*, among others.

The researchers caution, however, that they don’t yet understand why some children recover and others don’t, and have no way to predict which patients will do well. Further studies are planned to better understand the well-being and social and communication skills of those children who do “recover.”

According to Dr. Schultz, this study “provides an important scientific opportunity to study why and how these particular children did so well — what is it about their past interventions, their initial profile of strengths and weaknesses and their brain that we can learn from in order to try to generalize this result to the great majority of youth who don’t achieve this optimal outcome?”

To learn more about the Center for Autism Research’s extensive body of work, see the Center’s website.

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**Dr. Lamia Barakat to Head Section on Behavioral Oncology**

Frank Balis, M.D., and John M. Maris, M.D., recently announced the appointment of Lamia P. Barakat, Ph.D., to head the Section on Behavioral Oncology. Dr. Barakat is currently the Director of Psychosocial Services for the Division of Oncology, and she will be promoted to the rank of Professor of Clinical Psychology in the Department of Pediatrics effective July 1st 2013.

Dr. Barakat has an active and highly productive multidisciplinary research program focused on evidence-based assessment of psychosocial risk in families of children newly diagnosed with cancer, family interventions to improve disease management, quality of life, and school functioning in pediatric hematology and oncology, identifying strategies to increase recruitment and retention in pediatric clinical trials, and health care disparities in pediatric oncology follow-up care and transition. Dr. Barakat is also an outstanding teacher and mentor, and she leads the Cancer Center’s clinical psychology services, Drs. Balis and Maris noted.

The mission of the Section on Behavioral Oncology of the Center for Childhood Cancer Research is to apply behavioral translational research to improve outcomes in pediatric cancer across the continuum of care with an emphasis on neurodevelopmental and psychosocial processes of risk and resilience. Under Dr. Barakat’s leadership the section will continue to be highly productive in the clinical and research arenas and will continue to train the next generation of leaders in the field, Drs. Balis and Maris said.
CHOP Researchers Find Gene Variants Affect Human Lifespan

By broadly comparing the DNA of children to that of elderly people, gene researchers at The Children’s Hospital of Philadelphia have identified gene variants that influence lifespan, either by raising disease risk or by providing protection from disease.

“This research is the first genome-wide, population-based study of copy number variations in children associated with human longevity,” said Hakon Hakonarson, M.D., Ph.D., director of the Center for Applied Genomics at The Children’s Hospital of Philadelphia. Dr. Hakonarson led the study which appeared recently in PLOS ONE.

Copy number variations (CNVs) are losses or gains in DNA sequence that are usually rare, but which can play an important role in raising or lowering the risk of disease. The study team compared the rates of CNVs in a sample of 7,313 young subjects, 18 years old and below, from the Children’s Hospital network, to a group of 2,701 Icelandic subjects, 67 years old or above, recruited by the Icelandic Heart Association. The researchers used microchip arrays to perform the whole-genome CNV analyses.

“Our assumption was that CNVs appearing in children but not in the elderly were more likely to be disease-causing, while CNVs that were proportionately higher in older people were more likely to be protective, allowing them to live longer,” said Dr. Hakonarson.

Multiple sclerosis (MS) is an often debilitating chronic disease of the central nervous system in which myelin — the fatty sheath that insulates the nerves — is damaged, leading to nerve signaling loss. Symptoms of MS include numbness, weakness in the limbs, vision problems, and progressive disability. MS affects approximately 90 out of 100,000 people, and is mainly seen between the ages of 20 and 40. Pediatric MS is less common, representing perhaps 5 percent of all MS patients.

There is no cure for MS. While a variety of drugs exist to manage the disease’s symptoms and to slow its progression, current treatments are only effective up to a certain point, Dr. Grinspan pointed out.

After receiving her Ph.D. in biology from the University of Pennsylvania and completing a postdoctoral fellowship at CHOP under David Pleasure, M.D., Dr. Grinspan joined CHOP’s faculty in 1989. Including her postdoc, she has been conducting multiple sclerosis-related research since 1986, she said.

Much of Dr. Grinspan’s research has been devoted to better understanding oligodendrocytes, cells of the central nervous system that produce myelin, which dates to her time working with Dr. Pleasure. She originally began researching MS because Dr. Pleasure needed an expert in primary tissue culture, and found the work his lab was doing on oligodendrocytes and myelination (the development of the myelin sheath) to be “a good fit for my interests in combining basic cell biology and understanding of disease,” she said.

“MS is an interesting disease, and also it’s a significant disease,” Dr. Grinspan noted. In addition, the research performed by her team has the possibility to be transitional, she added.

Her work on oligodendrocytes recently led to a collaboration with neonatologist Rebecca Simmons, M.D., examining intrauterine growth restriction (IUGR), a relatively common complication of pregnancy. The researchers looked at the process of oxidative stress — an imbalance between free radicals and free radical scavengers that is commonly associated with prematurity and IUGR. Oxidative stress is also present in multiple sclerosis, and is thought to damage oligodendrocytes in much the same way.

In their search for ways to treat oxidative stress, the researchers examined the impact bone morphogenetic proteins (BMP) have on the development of oligodendrocytes. Previous work by Drs. Grinspan and Simmons showed that BMP inhibits oligodendrocyte maturation, so the researchers used cell culture and animal models to show that oxidative stress arrests myelination through BMP.

If BMP signaling could be arrested in fetuses or newborns or in adults with MS, the hope is that the development of diseases like cerebral palsy or MS could be stopped in their tracks.

Overall, Dr. Grinspan’s work wouldn’t be possible without funding from organizations like the Multiple Sclerosis Society. “I am honored to have won this award and am thankful that the MS society has enabled me to do this research through their continued support,” Dr. Grinspan said.
The Human Genome —Yep, There’s an App for That!

The app, called Genome Wowser, allows users to traverse the entire human genome — just like planning a travel route on Google Maps. Since its launch, the app has been consistently ranked as a top 10 “What’s Hot” medical app on iTunes, downloaded and updated thousands of times by researchers around the world.

Genetic Engineering & Biotechnology News recently named Genome Wowser one of the Best Science Apps available.

The app can find a searched-for gene on one of the 23 human chromosomes, displaying an interactive image of its precise location among the genome’s 3 billion base pairs. Genome Wowser can also tell users about a gene's known or suspected biological functions, identified mutations and variants of each gene, or learn how a gene's functions are modified when chemicals attach or separate from exposed sections of DNA.

“With this app, researchers can now access genomic data from anywhere with minimal effort, and they can immediately explore the genome visually by using the intuitive screen touches and gestures that have made the iPad® platform so powerful,” says CBMi director Peter White, Ph.D.

Want to know more about Genome Wowser? Check out the story in our most recent Research Annual Report, or visit the app's official Facebook page.

Extending CPR Saves Lives, National Studies Find

The Children's Hospital of Philadelphia researchers contributed to two recent studies showing that extending cardiopulmonary resuscitation (CPR) longer than previously thought useful saves lives in both children and adults. The research teams analyzed the impact of duration of CPR in patients who suffered cardiac arrest while hospitalized.

“These findings about the duration of CPR are game-changing, and we hope these results will rapidly affect hospital practice,” said Robert A. Berg, M.D., chief of Critical Care Medicine at Children's Hospital. Dr. Berg is also chair of the Scientific Advisory Board of the American Heart Association's Get With Guidelines-Resuscitation program (GWTG-R), a national registry that tracks and analyzes the resuscitation of patients after in-hospital cardiac arrests.

The investigators reported data from the GWTG-Resuscitation registry of CPR outcomes in thousands of North American hospital patients in two landmark studies, one of which was focused on children.

Dr. Berg was a co-author of the pediatric study, which was published recently in Circulation. After analyzing the hospital records of 3,419 children in the U.S. and Canada from 2000 through 2009, the investigators found that among children who suffered in-hospital cardiac arrest, more children than expected survived after prolonged CPR. Of those children who survived prolonged CPR — which is defined as lasting longer than 35 minutes — over 60 percent had good neurologic outcomes.

The conventional thinking has been that CPR is futile after 20 minutes, but these results challenge that, said Dr. Berg.

Another of the Circulation study’s co-authors, Vinay M. Nadkarni, M.D., a critical care and resuscitation science specialist at Children’s Hospital, noted that illness categories affected outcomes, with children hospitalized for cardiac surgery having better survival and neurological outcomes than children in all other patient groups.

The pediatric results parallel those found in the second GWTG-R investigation, which examined 64,000 adult patients with in-hospital cardiac arrests between 2000 and 2008. Dr. Berg also was also a co-author of that study, published in The Lancet in October 2012.

That study showed patients at hospitals in the top quartile of median CPR duration (25 minutes), had a 12 percent higher chance of surviving cardiac arrest, compared to patients at hospitals in the bottom quartile of median CPR duration (16 minutes). Survivors of prolonged CPR had similar neurological outcomes to those who survived after shorter CPR efforts.

The American Heart Association and American Stroke Association named the Lancet study as the top finding of the year in heart disease and stroke research in its annual list of major advances. Going forward, CPR researchers will seek to identify important risk and predictive factors that determine which patients may benefit most from prolonged CPR, and when CPR efforts have become futile.

“Taken together, the adult and pediatric results present a clear and hopeful message: persisting longer with CPR can offer better results than previously believed possible,” concluded Dr. Berg.
Required Certification for Clinical Research Coordinators

Clinical Research Coordinators (CRCs) are an essential component of the clinical research enterprise at CHOP, serving a pivotal role in human subjects protection and overall study management. The CHOP Research Institute (CRI) is committed to providing the proper education, training, and support for the CRCs. Toward this goal, the CRI is implementing a CRC certification program.

All Clinical Research Coordinators (CRCs) will be required to complete the Clinical Research Coordinator Certification program offered by the Office of Clinical and Translational Research (OCTR), according to the timeline described below. This requirement applies to any research staff performing research coordinator functions and/or listed in the role of study coordinator on an IRB protocol submission. Specific job titles may include, but are not limited to: Research Assistant, Research Coordinator, Clinical Research Coordinator, Research Nurse, Clinical Research Nurse, Study Nurse, and Clinical Project Manager.

Requirements for CRC Certification:

1. Certification in the CHOP institutional human subjects’ protections training (CITI training)
2. Completion of the CHOP on-line Study Coordinator Modules
3. Participation in the OCTR two day CRC training program
4. Participation in an additional three workshops/training programs related to human subjects research offered by one of CHOP’s administrative departments, such as the IRB, Office of Research Compliance and Regulatory Affairs, Office of IND/IDE Support or the Clinical Trials Office.

Timelines:

Consistent with current policies, all CRCs must complete CITI training and the on-line Study Coordinator Modules prior to participating on an IRB approved protocol. Current CRCs are required to complete the OCTR two-day training program and the three CHOP-sponsored human subject research workshops by June 1, 2015. All CRCs hired on or after June 1, 2013 must complete the two-day training program and three workshops within 24 months of their hire. The first two day training session will be offered in March, 2013 and then every two months thereafter.

Information on registration in Learning Link will be forthcoming. Questions about this new requirement may be addressed to the Office of Clinical and Translational Research: navigator@email.chop.edu.