

Bench to Bedside



RESEARCH AT THE CHILDREN'S HOSPITAL OF PHILADELPHIA

August 2009

New Map of Genomic Variation Will Assist Disease Research

Genetics researchers have unveiled a powerful new resource for scientists and health providers studying human illnesses – a reference standard of DNA deletions and duplications found in the human genome. Drawn from more than 2,000 healthy persons, the study provides one of the deepest and broadest sets of copy number variations (CNVs) available to date, along with a new research tool for diagnosing and identifying genetic problems in patients.

A team from The Children's Hospital of Philadelphia published its high-resolution map and analysis of CNVs in the human genome in the July 10 online edition of the journal *Genome Research*.

In contrast to alterations to a single base of DNA, which are single nucleotide polymorphisms (SNPs), often referred to as “snips,” CNVs are larger variations in DNA structure. As changes to a single DNA letter, SNPs might be considered misspellings or alternate spellings of a word, while CNVs are losses or repetitions of whole phrases, paragraphs, or even pages. Some CNVs are inserted stretches of DNA from other parts of the genome. Both SNPs and CNVs contribute to genetic diversity and disease by changing the action of genes for which DNA carries coded instructions.

“We all carry a number of these variations in our own genomes,” says study co-leader Peter S. White, Ph.D., a molecular geneticist and director of the Center for Biomedical Informatics at Children's Hospital. “Some CNVs contribute to a disorder, but most of them do not, and it is often challenging to determine which are important. One approach is to compare CNVs in healthy individuals to those in patients with a disease, to find those CNVs that seem to occur primarily in people with a certain disease. Our map provides a large and uniform baseline standard to indicate which CNVs represent normal variation.”

The investigators analyzed DNA from blood samples taken from 2,026 subjects. The subjects were healthy children and their parents, all drawn from primary care and well-child clinics in the Children's Hospital healthcare network. Of the samples, 65 percent were from Caucasians and 34 percent from African-Americans.

The number of subjects makes this CNV collection among the largest reported to date, and because all the samples were collected and analyzed under the same protocols, using the same technology, and at one institution, the results have a uniformity that increases their value as benchmarks. To detect CNVs in the thousands of samples, the investigators used highly automated gene-analyzing technology at the Center for Applied Genomics at Children's Hospital, directed by Hakon Hakonarson, M.D., Ph.D., a co-leader of this study.

The CNV map has a higher resolution than most previous efforts, say the authors, with more than 50,000 CNVs catalogued throughout

the genome. Three-quarters of these were “non-unique,” occurring in multiple unrelated individuals. More than half of these non-unique CNVs were newly discovered. On average, the healthy subjects in the study have approximately 27 CNVs each.

The investigators have posted the full CNV database on the Hospital's Web site at <http://cnv.chop.edu/>, where it is freely available in searchable form to gene researchers worldwide. The Web browser also enables researchers to compare specific CNVs to those collected in public data repositories from other institutions.

“This resource will be very important in enabling rapid and accurate diagnoses of rare diseases resulting from CNVs,” says lead author Tamim H. Shaikh, Ph.D., a molecular geneticist at Children's Hospital. Often puzzling to physicians, such genetic diseases may be individually rare, but collectively occur at frequencies that are comparable to the incidence of well-known disorders such as Down syndrome.

As an example of the clinical usefulness of their database, the authors analyzed DNA from a child with multiple congenital problems, including developmental delay and brain malformations. They found 35 CNVs, of which 32 were previously detected in healthy controls. Two of the patient's three unique CNVs were relatively small in size, but the third CNV was a deletion in chromosome 17 that encompassed 51 genes, including several that are active in early prenatal development. Unlike most of the other CNVs, it did not occur in the child's parents, strongly supporting the conclusion that the chromosome deletion arose spontaneously in the patient and that it caused the child's disease.

The new database has another strength, adds Dr. Shaikh. Because it analyzed large numbers of samples from both Caucasians and African-Americans, it measured CNV levels that differ between the two ethnic groups, and enables clinicians to make more precise diagnoses. Dr. Shaikh adds that the researchers expect to expand the database with larger sample sizes and data from additional ethnic populations.

In addition to its use in diagnosis, says Dr. White, the database may also assist researchers studying molecular evolution, for example, those investigating how genetic variations occurred as human populations spread across continents.

Funds from the National Institutes of Health, the Pennsylvania Department of Health, and the Cotswold Foundation supported this research. In addition, the David Lawrence Altschuler Chair in Genomics and Computational Biology at Children's Hospital contributed funds to the study. Co-authors, including co-lead author Xiaowu Gai, represented both Children's Hospital and the University of Pennsylvania School of Medicine.

New Research Employees (June 2009)

We welcome the following new research employees:

Administrative Director – IND/IDE Support Alice Pine	Research Associates Eric Hanson Jason Mills
Adolescent Health Educator Yexsy Alicea	Research Coordinators Sage Green Rachel Myers Alisa Reznikov
Analyst/Programmers Byron Ruth Salim Zayat	Research Data Analyst Harini Eavani
Clinical Assistant/Driver Wayne Tschanz	Research Nurse Kay Isola
Clinical Research Assistants Kira Carter Tara Esposito Janelle Letzen Joseph Mechak James Taylor	Research Project Engineer Caitlin Locey
Clinical Research Coordinators Theresa Kerbowski Rosa Kim Jena Lilly Jennifer Schneider	Research Technicians Richard Adamo Jr. Rachel Bastian Lili Belcastro Alan Chiu Emily Dudek Shweta Dutta Heather Fetting Grady Hedstrom Chase Hulderman Jose James Haein Kim Fabienne Kyle Paul Le Jenna Maurer Almedia McCoy Monica Murphy Kaitlin Murray Baali Musisi Muganga Geoffrey Norris Laura Quick Erik Walp Roisin Walshe Zhen Yo Zheng
Clinical Nurse Gwenn LaRagione	
Clinical Research Nurse Coordinator Allison Paisley	
Counselor Megan Bugbee	
Director – Human Subjects Research Amy Schwarzhoff	
Lab Assistant Alexander Moy	Resource Coordinators Stephanie Shealy Jonathan Stem
Large Animal Research Support Specialists Nichole Hall Nicole Waltemire	Scheduler/Patient Service Representative Maria Lopez
Office Coordinator Michelle Smith	Small Animal Research Support Specialist Walter Johnson
Research Assistants Iris Insogna Divya Prasad Marlon Satchell Nina Somwaru	Social Worker Kathleen Whitfield Technical Director Xianqun Luan

ADHD Genes Found; Known to Play Roles in Neurodevelopment

Pediatric researchers have identified hundreds of gene variations that occur more frequently in children with attention-deficit hyperactivity disorder (ADHD) than in children without ADHD. Many of those genes were already known to be important for learning, behavior, brain function, and neurodevelopment, but had not been previously associated with ADHD.

“Because the gene alterations we found are involved in the development of the nervous system, they may eventually guide researchers to better targets in designing early interventions for children with ADHD,” says lead author Josephine Elia, M.D., a psychiatrist and ADHD expert at Children’s Hospital.

The study appeared online June 23 in the journal *Molecular Psychiatry*.

The alterations examined in the study, copy number variations (CNVs), are missing or repeated stretches of DNA. CNVs have recently been found to play significant roles in many diseases, including autism and schizophrenia. Everyone has CNVs in their DNA, but not all of the variations occur in locations that affect the function of a gene. The current study is the first to investigate the role of CNVs in ADHD.

ADHD is the most common neuropsychiatric disorder in children, affecting an estimated 1 in 20 children worldwide. It may include hyperactive behavior, impulsivity, and inattentive symptoms, with impaired skills in planning, organizing, and maintaining focus. Its cause is unknown, but it is known from family studies to be strongly influenced by genetics.

Drawing on DNA samples from the Children’s Hospital pediatric network, the researchers analyzed genomes from 335 ADHD patients and their families, compared to more than 2,000 unrelated healthy children. The team used highly automated gene-analyzing technology at the Center for Applied Genomics at Children’s Hospital, directed by Hakon Hakonarson, M.D., Ph.D., a co-leader of this study.

The study team found a similar quantity of CNVs in both groups. However, distinct patterns emerged. Among 222 inherited CNVs found in ADHD families but not in healthy subjects, a significant number were in genes previously identified in other neurodevelopmental disorders, including autism, schizophrenia, and Tourette syndrome. The CNVs found in ADHD families also altered genes important in psychological and neurological functions such as learning, behavior, synaptic transmission, and nervous system development.

“We took a systems biology approach, putting genes into groups with common functions,” says study co-leader Peter S. White, Ph.D., a molecular geneticist and director of the Center for Biomedical Informatics at Children’s Hospital. “We found that the sets of genes more likely to be changed in ADHD patients and families affected functions that made sense biologically.” For instance, says Dr. White, the team found four deletions of DNA in a gene recently linked to restless legs syndrome, a type of sleep disorder common in adults with ADHD.

Another deletion occurred in a gene for a glutamate receptor. Glutamate is a neurotransmitter, a protein that carries signals in the brain. While ADHD medications act on dopamine and serotonin, which are also neurotransmitters, this new finding may suggest an important role for glutamate as well, at least for some ADHD patients.

Some of the biological pathways involved in ADHD may also be common to other neurological conditions, say the investigators. Likewise, there is some overlap among the CNVs found in ADHD that also occur in autism, schizophrenia, and other neurological disorders. This overlap was not surprising, says Dr. Elia, because ADHD patients frequently also have one of more of these disorders. However, as researchers learn more about specific genes in neurological conditions, the hope is that researchers might in the future personalize treatments to a patient’s own genetic profile, to achieve more targeted, specific therapies.

Drs. Elia and White stressed that much further work must be done before genetic findings lead to ADHD treatments.

The National Institutes of Health provided grant support for the study, as did the University of Pennsylvania, the Pennsylvania Department of Health, the Cotswold Foundation, and the ADHD: Climbing to a Cure Foundation. Drs. Elia, White and Hakonarson all are faculty members of the University of Pennsylvania School of Medicine. Xiaowu Gai, Ph.D., of the Center for Biomedical Informatics at Children’s Hospital, was a co-first author with Dr. Elia. Other collaborators were from Children’s Hospital and Penn.

More Gene Mutations Linked to Autism Risk

More pieces in the complex autism inheritance puzzle are emerging in the latest study from a research team including geneticists from Children's Hospital, the University of Pennsylvania School of Medicine, and several collaborating institutions. This study identified 27 different genetic regions where rare copy number variations – missing or extra copies of DNA segments – were found in the genes of children with autism spectrum disorders (ASDs), but not in the healthy controls. The complex combination of multiple genetic duplications and deletions is thought to interfere with gene function, which can disrupt the production of proteins necessary for normal neurological development.

“We focused on changes in the exons of DNA – protein-coding areas in which deletions or duplications are more likely to directly disrupt biological functions,” says study leader Hakon Hakonarson, M.D., Ph.D., director of the Center for Applied Genomics at Children's Hospital. “We identified additional autism susceptibility genes, many of which, as we previously found, belong to the neuronal cell adhesion molecule family involved in the development of brain circuitry in early childhood.” He adds that the team discovered many “private” gene mutations, those found only in one or a few individuals or families – an indication of genetic complexity, in which many different gene changes may contribute to an ASD.

“We are finding that both inherited and new, or *de novo*, genetic mutations are scattered throughout the genome and we suspect that different combinations of these variations contribute to autism susceptibility,” says co-author Maja Bucan, Ph.D., professor of genetics at the University of Pennsylvania School of Medicine and chair of the steering committee for Autism Speaks' Autism Genetic Resource Exchange (AGRE).

The researchers compared genetic samples of 3,832 individuals from 912 families with multiple children with ASDs from the AGRE cohort against genetic samples of 1,070 disease-free children from Children's Hospital. This study also uncovered two novel genes in which variations were found, *BZRAP1* and *MDGA2*, thought to be important in synaptic function and neurological development, respectively. Interestingly, key variants of these genes were transmitted in some, but not all, of the affected individuals in families.

The findings were published in the June 26 edition of the journal *PLoS Genetics*.

By further refining the genetic landscape of ASDs, the current study expands the findings of two large autism gene studies published in April. One study was the first to report common gene variants in ASDs. The other identified copy number variants that raise the risk of having an ASD. Both studies found gene changes on two biological pathways with crucial roles in early central nervous system development. Drs. Hakonarson and Bucan said the latest findings reinforce the view that multiple gene variants, both common and rare, may be interacting to cause the heterogeneous group of disorders included under autism spectrum disorders.

AGRE provided genetic biomaterials and clinical data from families having more than one member diagnosed with an ASD. Blood samples donated by children and their families at Children's Hospital were used as healthy controls. AGRE makes data publicly available to qualified researchers worldwide.

Investigator Receives HHMI Early Career Award

Edward Behrens, M.D., Division of Rheumatology, received an Early Career Physician-Scientist Award from the Howard Hughes Medical Institute (HHMI) for his research on how the immune system provokes autoimmune diseases by attacking its own tissues.

Dr. Behrens, an alumni of the HHMI Medical Fellows Program, focuses his research on dendritic cells, which initiate the immune system's response to foreign invaders and can also trigger autoimmune or autoinflammatory disorders if improperly activated. His research has led to discoveries about how the immune system malfunctions in children who have juvenile arthritis and other autoimmune diseases. He continues to investigate proteins called toll-like receptors inside dendritic cells and to develop computational tools to improve healthcare information about patients with autoimmune diseases.

Dr. Behrens is one of 11 promising physician-scientists from across the country who received the award to support work on a variety of pressing research questions. The award provides recipients

with a \$375,000 grant, distributed over the next five years, to help them launch and develop innovative research programs at a critical stage of their careers.

Physician-scientists in their first permanent academic position often experience pressure to spend more time in the clinic and less time doing research, and lack sufficient funding to collect data for an effective grant proposal. The Early Career Physician-Scientist Award provides funding to accelerate data collection and analysis and requires that awardees spend at least 70 percent of their time doing research.

The Early Career Physician-Scientist Award is designed as a way to help investigators get their first NIH grant, with the hope it is a step in a long career of research that influences public health. HHMI is dedicated to increasing the number of researchers who are able to translate basic science discoveries into improved treatments for patients.

Oncology Leaders Named at The Children's Hospital of Philadelphia

Children's Hospital has announced the appointments of two nationally prominent pediatric oncologists to leadership positions in the Hospital's Division of Oncology.

John M. Maris, M.D., has been named chief of the division, and Frank M. Balis, M.D., has joined the Hospital as director of clinical research for the division. Dr. Balis also was named director of clinical research for the Hospital's Center for Childhood Cancer Research, directed by Dr. Maris.

A member of the Oncology Division staff since 1995, Dr. Maris became acting chief of the division in 2008 and holds the Giulio D'Angio Endowed Chair in Neuroblastoma Research. He is an associate professor of pediatrics at the University of Pennsylvania School of Medicine, where he is also a member of the Abramson Family Cancer Research Institute.

Dr. Maris is an expert on the molecular genetics and treatment of neuroblastoma, the most common solid cancer in children. In 2008, he led an international team that identified for the first time common DNA variants that are the genetic origin of neuroblastoma. He is the principal investigator of numerous National Institutes of Health-funded grants focused on the disease, and also chairs the Neuroblastoma Disease Committee of the Children's Oncology Group, a national cooperative research organization.

As Oncology Division chief, Dr. Maris succeeds Garrett M. Brodeur, M.D., also a nationally prominent expert in neuroblastoma, who remains in the division as an attending physician and researcher.

Dr. Maris notes that "Dr. Brodeur led the division through a decade-long period of dramatic expansion, with tremendous growth in both the clinical and research programs."

Dr. Balis comes to Children's Hospital from a 27-year career at the National Cancer Institute, where he was most recently the Institute's clinical director. His research program has focused on drug development for childhood cancers. He is most prominent for his seminal contributions to intrathecal administration, a method of delivering anticancer drugs directly to the spinal fluid that has allowed doctors to abandon the use of craniospinal radiation in treating most children with acute lymphoblastic leukemia.

Dr. Maris, who led the effort to recruit Dr. Balis to Children's Hospital, says, "As we seek to re-engineer how we develop drugs for children with cancer, we recognized the need for a national leader in the area of clinical research to spearhead our efforts. It became clear very quickly that Dr. Balis was the ideal candidate." In this newly created position, Dr. Balis will oversee all aspects of clinical research on pediatric cancer.

Children's Hospital cares for more children with cancer than any other general pediatric hospital in the United States. Dr. Maris adds that Dr. Balis will expand existing strong clinical research programs in neuroblastoma, brain tumors, and leukemia, while establishing additional clinical research programs in pediatric sarcomas and lymphomas.

Changes to Conflict of Interest Policy Address Consulting Agreements

Children's Hospital is steadfast in its commitment to conduct its affairs in accordance with the highest ethical and legal standards. To maintain these standards, the Hospital has instituted policies dealing with potential, perceived, and actual conflicts of interest in the hope of avoiding conflicts.

One such area of potential conflict of interest rests with consulting agreements between investigators and industry. In recent months the media and the federal government have increased their scrutiny into relationships between companies and academic physicians and investigators. In this environment it is critical that Children's Hospital join other institutions, including the University of Pennsylvania, in managing these relationships and minimizing perceived or actual conflicts of interest.

Effective July 1, 2009, Children's Hospital requires that all outside consulting activities, whether paid or unpaid, be reviewed and approved prior to engagement. Consulting activities could include, but are not limited to, data safety monitoring boards and advisory boards.

This change applies to all Hospital scientists, including those on the Hospital's medical staff, and others who are faculty members at the University of Pennsylvania and perform some or all of their duties under the auspices of Children's Hospital. Review and approval for outside consulting activities by other employees remains optional.

The Conflicts of Interest, Patent, and Intellectual Property policy and Interactions With Vendors policy have been modified to reflect this change in practice.

The Office of Technology Transfer (OTT) is the contact point to initiate the review process, please send consulting agreements to techtransfer@email.chop.edu. For further information, please contact Debbie Schmidt at Schmidt@email.chop.edu or Ellen Purpus at Purpus@email.chop.edu, or visit the OTT intranet site at <https://intranet.research.chop.edu/display/depttech/Home>.

New Research Workshop Series on Conducting Research at CHOP Announced

The Office of Responsible Research Training has launched “Conducting Research at CHOP,” a new workshop series designed to educate the Children’s Hospital workforce on key topics related to the implementation and management of sponsored research.

Sessions will be held quarterly and will focus on navigating research administration, best practices in managing grants and budgets, conducting clinical trials, and ensuring the safe and ethical conduct of research.

The first session will be held Sept. 15 in Abramson Center, Room 123-C from 10 a.m. to noon and will concentrate on submitting a proposal at CHOP. The presentation will be given by Berenice Saxon, Collette Ryder-Consugar, and Julia Wagner of Sponsored Projects and Research Business Management.

Developing successful research proposals is both an art and a science. This session offers practical advice and guidelines for successfully preparing and submitting your proposal to external funding agencies. The series is open to all Hospital personnel who have responsibilities or interests related to the administration and management of sponsored research.

Please note that seating is limited and registration is required.

To learn more about the series and to register for the first session visit: <https://intranet.research.chop.edu/display/deptrrt/Seminars+#Seminars-ConductingResearchatCHOP>.

For additional information, contact Janet Stuart at stuartj@email.chop.edu or 267-426-7496.

Bioinformatics Core Now Recovering Costs on Hourly Basis

The Bioinformatics Core Facility (BiC) supports the varied needs of the Research Institute with tiered bioinformatics assistance, providing efficient and quality service to investigators and research staff.

BiC recently modified its funding options to allow recovery of costs on an hourly basis in addition to its percent effort-based funding. The new hourly recovery option began July 1.

Initial consultations with a BiC staff member or director remain free of charge, and investigators may

still designate a percent effort on funded grants for one or more BiC staff members.

Under the latest approach, investigators may opt to reimburse the core at \$58 per hour if no BiC staff member is funded by the project. This hourly rate applies only to Children’s Hospital investigators.

If you have any questions regarding BiC services, please contact Xiaowu Gai, Ph.D., at gai@email.chop.edu or ext. 6-7023.

HAVE NEWS?

Contact Jennifer Long at ext. 4-2105
or by e-mail at longj@email.chop.edu.

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