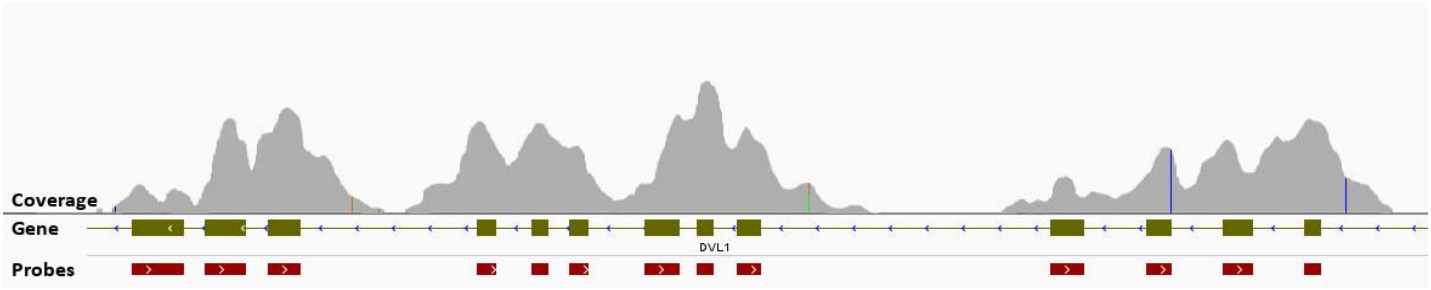
Duke University Medical Center

News About...

Neural Tube Defects Research

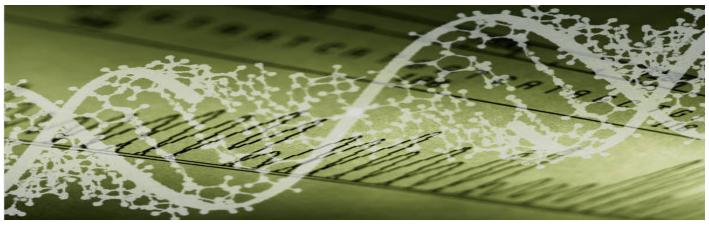
Summer 2014

Genetic Risk for Anencephaly Proves to be Complex



Humans have approximately 25,000 different genes. Genes are made of DNA. We all have DNA changes within our genes that make us unique from other people. Most DNA changes are not harmful but some DNA changes can cause medical conditions. Changes within genes are thought to contribute to anencephaly and other neural tube defects. In recent years, it has become possible to read or sequence all the genes in a person (exome sequencing). In order to comprehensively investigate genes involved in anencephaly, we performed exome sequencing in a family that experienced three pregnancies with anencephaly. This family was selected for exome sequencing as we felt with three affected pregnancies, there was likely a genetic factor involved.

Exome sequencing was completed on DNA samples from all three pregnancies with anencephaly, both parents and one healthy sibling. We expected to find one or two DNA changes in all three affected pregnancies that were not present in the healthy sibling. However, what we actually found was a more complex interaction of 25 DNA changes. All three pregnancies with anencephaly had more of these DNA changes than the healthy sibling. Though not what we originally expected, these results are consistent with the idea that multiple genetic factors contribute to anencephaly.

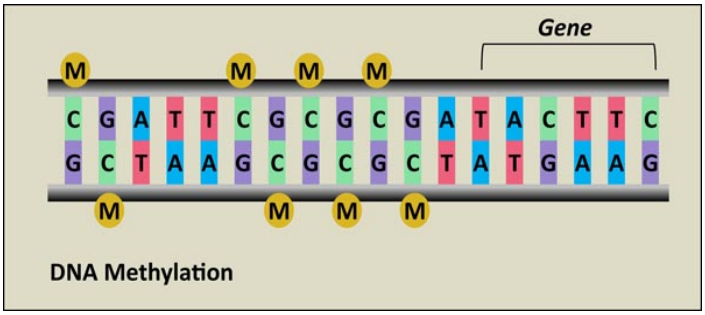


IN THIS ISSUE:

Twins Help us Look Outside the Genes

While DNA changes are important in the risk of developing anencephaly, epigenetic changes are also believed to play a role. The field of epigenetics studies chemicals or markers that sit on top of the DNA that regulate gene expression (switch genes on and off). To investigate the importance of epigenetic changes in anencephaly we compared epigenetic profiles of identical twins, one with anencephaly and one without. Identical twins have the same DNA; therefore differences between identical twins are more likely to be caused by changes in epigenetic markers.

Overall, the epigenetic profiles of the twins were quite similar. However, we identified over 1,000 sites within the genome of the twins where there were epigenetic differences between the twins. One of the most notable differences is a cluster of sites that may control genes that are involved in neural tube closure. We are currently expanding our analysis of these sites in family members of the identical twins and also in pairs of non-identical twins.



http://www.delawareneuroscience.org/Pages/Roth.htm

Collaborative Research into Fumonisin Continues

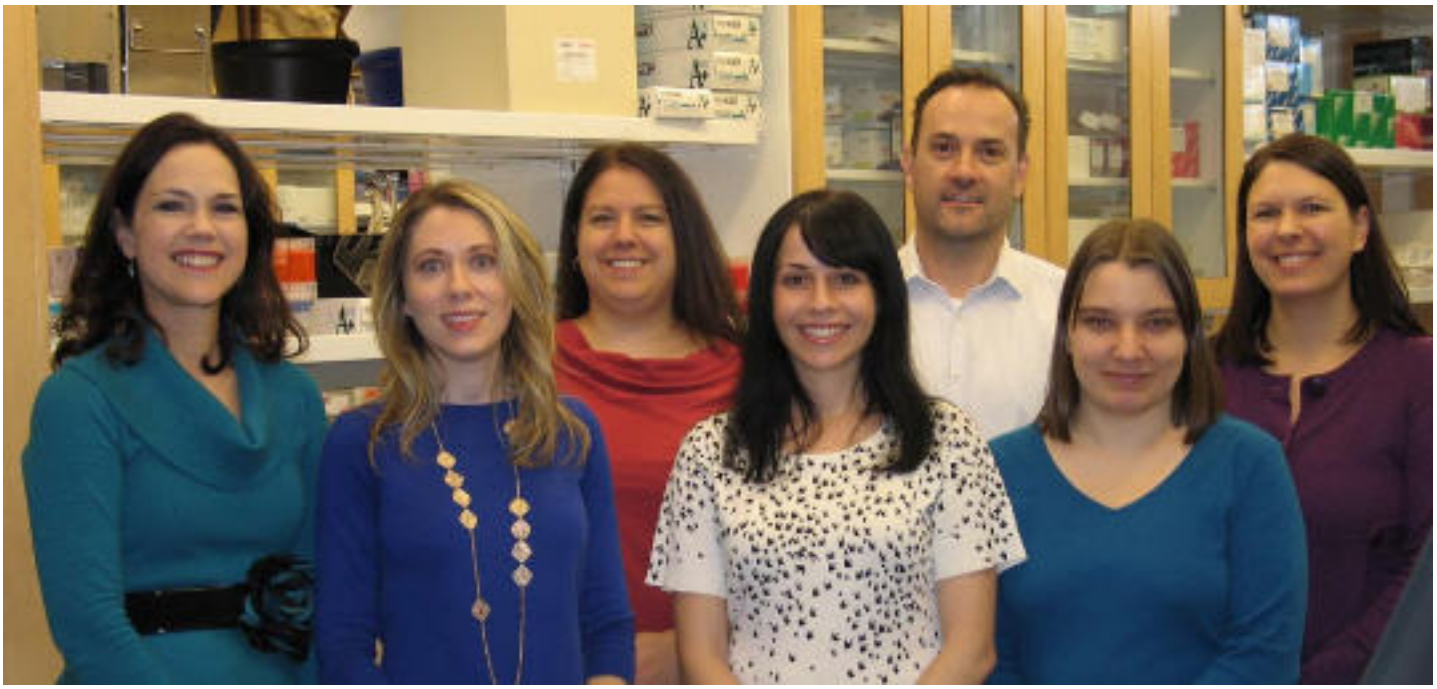
Fumonisin is a toxin produced by a mold that grows on corn in warm, relatively dry climates. It has been shown to cause NTDs in mice, and is thought to contribute to higher numbers of babies born with NTDs in areas where corn is eaten often. In collaboration with researchers from DX Molecular in Guatemala, Creighton University and the U. S. Department of Agriculture (USDA), we are working to investigate fumonisin's impact on the people exposed to it.


Dr. Gregory in Guatemala

Our collaborators in Guatemala have collected over 1,700 blood samples from healthy Guatemalan women of childbearing age. The samples will be analyzed to determine how much fumonisin is present in the blood. We know that fumonisin exposure impacts a particular biologic pathway called the "sphingolipid" pathway. Currently, our team is looking for DNA changes in the sphingolipid pathway genes to determine if these genes influence the levels of fumonisin that are in the blood. In addition, we will look for changes in gene expression (genes turned on or off) that will tell us if different levels of fumonisin impact the way our genes function.

At the same time, our collaborators at Creighton University are working with two different types of mice, one type which develops NTDs when given fumonisin (sensitive type), and another type that does not. Our lab performed exome sequencing (reading all the genes) in both types of mice. Genetic differences were found in the sensitive type, which appear to contribute to fumonisin sensitivity.

Duke NTD Study Team



Left to right: Allison Ashley-Koch (Principal Investigator), Heidi Cope (Study Coordinator), Karen Soldano (Lab Research Analyst), Renee Leduc (Graduate Student), Simon Gregory (Principal Investigator), Deidre Krupp (Graduate Student), Melanie Garrett (Biostatistician)

Meeting Thomas's Colleagues: The Participants' Point of View

By Sarah Gray

On March 23, 2010, I waddled into the operating room at Fairfax Hospital in Falls Church, Virginia, holding a bag of test tubes and directions for extracting cord blood. Our identical twin boys, Thomas and Callum, were born and their cord blood was sent to Duke by FedEx within hours. Thomas died of anencephaly five days later. In addition to his cord blood, we donated Thomas's eyes and liver cells to other research programs. I imagined what the researchers thought about when they opened these packages. I wondered what they did with the donations. And I felt slightly jealous of the time they would be able to spend with my little guy. My time getting to know Thomas was over, but theirs was just beginning.

In 2012, I realized that nothing was stopping me from meeting these mysterious individuals who were a new part of my family's history. So I booked a hotel room. My husband, Ross, Callum and I drove five hours from Washington, D.C. to Duke to meet the anencephaly researchers on November 11, 2012. We learned the

Thomas Ethan Gray

names, shook the hands, and even hugged the people who received Thomas's donation: Allison Ashley-Koch, Simon Gregory, Deidre Krupp and Heidi Cope. We got the opportunity to learn about their work and ask questions. Karen Soldano gave us a tour of the lab where we followed the journey that Thomas's samples took. We visited the area where his sample is stored. It felt like visiting our son at college and meeting his new friends.

Meeting these researchers helped me understand that Thomas is relevant in this community. He has co-workers and colleagues, and they cannot do their job without him. My family has something common with these researchers: we both spend our time thinking and talking about Thomas – just in different ways.

Anencephaly Psychological Impact Study Begins

In 2012, the NTD study coordinator, Heidi Cope, received the Audrey Heimler Special Project Award from the National Society of Genetic Counselors (NSGC). This was a $5,000 award given to fund a one year research project to examine the psychological impact of losing a baby to anencephaly. The study began enrolling participants in March of 2013 and completed enrollment in early 2014. Questionnaires were mailed to 215 women and 177 men who lost a baby to anencephaly to measure symptoms of post-traumatic stress, grief and depression. In addition, information about demographics, social risk factors and pregnancy choices were also collected to determine if certain factors or choices contribute to better or poorer psychological outcome.

Ultimately, this research will assist healthcare providers in counseling their patients on the psychological impact of this type of loss. In addition, patient care recommendations will be developed to aid healthcare providers in providing appropriate information and resources to patients who receive a prenatal diagnosis of anencephaly.

Visiting Graduate Student Investigates CECR2

Renee Leduc is currently in the 5th year of her PhD program, which is being completed at the University of Alberta, Canada under the supervision of Dr. Heather McDermid. Dr. McDermid's research lab focuses on the study of a particular gene, CECR2. Changes in this gene can cause exencephaly in mice (equivalent to anencephaly in humans). Renee's research is directed towards identifying additional genes that, in combination with CECR2, contribute to exencephaly in mice. Her efforts have yielded a list of several interesting genes. Since mice and humans have fairly similar genes, she will examine CECR2 and the other genes of interest in our anencephaly participants in order to determine if any of these genes contribute to anencephaly in humans.

Eighth International Conference on NTDs

In October 2013, scientists and clinicians from the US, Europe, Central America and Australia descended on Austin, Texas for the biannual International Conference on NTDs. The scientists at the meeting had backgrounds in epidemiology, biology, embryology, genetics, neurosurgery and pediatrics. Information on NTD research, prevention and treatment was presented. The Duke NTD research team, represented by Dr. Simon Gregory and graduate student Deidre Krupp, presented much of the findings discussed in this newsletter. In the field of genetics and genomics the Duke team showed our continued leadership and we, like others scientists at the meeting, maintain a passion to identify the causes and cures of NTDs.

An award for best student paper is presented at each meeting in honor of the founder of the Duke NTD study, Dr. Marcy Speer, who passed away from breast cancer in 2007. The 2013 Marcy C. Speer Memorial Award recipients were Dr. Ran Blekhman from the University of Minnesota and Ms. Juliette Petersen from Howard Hughes Medical Institute.

Recent Publications

Nitric Oxide (NO) Genes and Neural Tube Defects

Nitric oxide genes have been hypothesized to play a role in neural tube closure. We examined three nitric oxide synthase genes (NOS1, NOS2 and NOS3) in 3,109 individuals from 745 families, which included individuals with many different types of neural tube defects (NTDs). Interaction between these genes and the MTHFR C677T polymorphism (previously implicated in NTD risk) was also examined. Evidence was found that all three NOS genes may contribute to the occurrence of NTDs.

While associations were found for all types of NTDs, the most significant associations were for cranial NTDs (anencephaly, acrania and encephalocele). NOS2 was associated with all cranial NTDs while NOS1 in combination with the MTHFR 677TT genotype was associated with anencephaly and acrania. This provides evidence that the NOS genes may play a role in causing NTDs and that different genes may contribute to different types of NTDs.

This paper was a finalist for the 2014 James G. Wilson Publication Award which is presented in recognition of the best paper published in the journal Birth Defects Research.

Soldano K., Garrett M., Cope H., Rusnak J., Ellis N., Dunlap K., Speer M., Gregory S., and Ashley-Koch A. (2013) Genetic association analyses of nitric oxide synthase genes and neural tube defects vary by phenotype. *Birth Defects Research (Part B),* 98:365-373.

Adults with Myelomeningocele

With improved medical care, increased numbers of individuals with myelomeningocele (MMC) are surviving into adulthood. However, little is known about

life as an adult with MMC. We collected information from 90 adults with MMC in an effort to learn more about their education, employment, relationships, reproduction and life satisfaction.

Many of the adults with MMC in the study were employed, lived independently and had partner relationships, which contributed to increased life satisfaction. The most consistent variable associated with difficultly attaining adult milestones was hydrocephalus, the presence of which reduced the likelihood of living independently, having a partner and having children.

Cope H., McMahon K., Heise E., Eubanks S., Garrett M., Gregory S., and Ashley-Koch A. (2013) Outcome and life satisfaction of adults with myelomeningocele. *Disability and Health J*, 6:236-243.

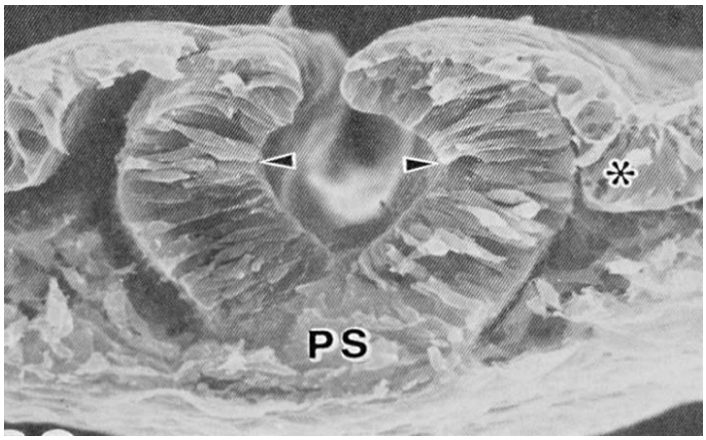
Investigating Genes Involved in Normal Neural Tube Closure

In order to aid the investigation into genes that cause neural tube defects (NTDs), we wanted to gain a better understanding of which genes are involved (turned on) in the formation of the normal neural tube. To do this, neural tissues were collected from early pregnancy terminations (performed at the request of the patient for reasons completely unrelated to this study) between 26 and 32 days of gestation.

We examined the genes turned on at different time periods (at the time of neural tube closure and after neural tube closure was completed) and also different regions of the neural tube (precursor of the brain and spinal cord). The genes involved in closure of the neural tube were similar for the regions of the brain and spinal cord. However, the genes involved varied at different time periods. Time appears to influence which genes are turned on in the developing central nervous system more than location. We therefore conclude that the genes involved in human neural tube closure are generally similar in both brain and spinal cord regions, and the turning on/off of genes is coordinated more by time rather than location. This indicates that the same genes may contribute to different types of NTDs.

This paper was a finalist for the 2013 James G. Wilson Publication Award which is presented in recognition of the best paper published in the journal Birth Defects Research.

Krupp D., Xu P., Thomas S., Dellinger A., Etchever H., Vekemans M., Gilbert J., Speer M., Ashley-Koch A., and Gregory S. (2012) Transcriptome profiling of genes involved in neural tube closure during human embryonic development using long serial analysis of gene expression (Long-SAGE). *Birth Defects Research (Part A)*, 94:683-692.



Enrollment Update

Due to funding limitations, study enrollment has been limited to anencephaly trios (baby with anencephaly plus both parents) for the last two years. In 2012 and 2013, 62 anencephaly trios joined the study. In total, we have collected DNA samples from 5,630 individuals in 1,485 families. Participating families come from all across the United States and Canada with all types of NTDs; however the majority of participants have either spina bifida or anencephaly.

Thank you to all the families who have participated over the years. Research toward a better understanding of the causes of neural tube defects would not be possible without your help.

Keep us Updated

Please keep us in mind when important changes happen in your family. We would like to know if there are any major changes in the health status of family members, such as if another family member is diagnosed with a neural tube defect or if you have had any additional pregnancies. Also, if you should move, please give us your updated contact information. You may give us your updates by calling us at **(919) 684-0655** or by sending an email to **heidi.cope@duke.edu**. Thank you!

In Loving Memory

We would like to express our sincere condolences to families who have lost a baby or other family member to a neural tube defect. We realize that this process is an extremely difficult one for your family. We appreciate your sharing with us such precious information about your loved-one and the time and effort you have taken to enroll in our research study. Please know that the memory of your family member is honored by your participation and we are truly grateful.

Follow us on Facebook

For periodic updates on anencephaly research being conducted at the Duke University Medical Center "like" us on Facebook at:
https://www.facebook.com/pages/Anencephaly-Study-at-Duke-University-Medical-Center/256402881048244

Who Pays for Research?

Conducting genetic research studies is painstaking and expensive work that relies on funding support from both public and private sources. Over the years the Duke NTD team has been awarded research funds or grants from March of Dimes, National Institute of Child Health and Human Development (NIH), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Environmental Health Sciences (NIEHS), and from private donors whose lives have been touched by neural tube defects.

We are often asked if we can accept donations to support the NTD research, sometimes in memory of a baby or loved one who has passed away. The answer is yes, and these donations are very much appreciated!

If you or someone you know would like to make a gift, we have created the Duke NTD Research Fund. To make a financial gift to NTD research, you may visit the secure web site of the Office of Alumni and Development Records at www.giftrecords.duke.edu and click on "Make a Gift Now!" and then "Make a Credit Card Gift." **Under Designations click on "Add an unlisted designation" and type in 3912359**. Or you can mail your tax-deductible donation to the address below:

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NTD Research Fund
Box 3445
Durham, NC 27710