Duke University Medical Center

News About...

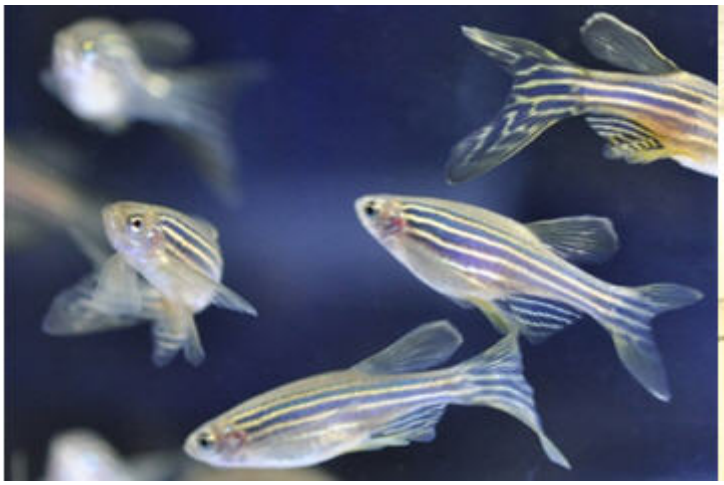
Neural Tube Defects Research

Spring 2016

Using Zebrafish to Help us Study Neural Tube Defects

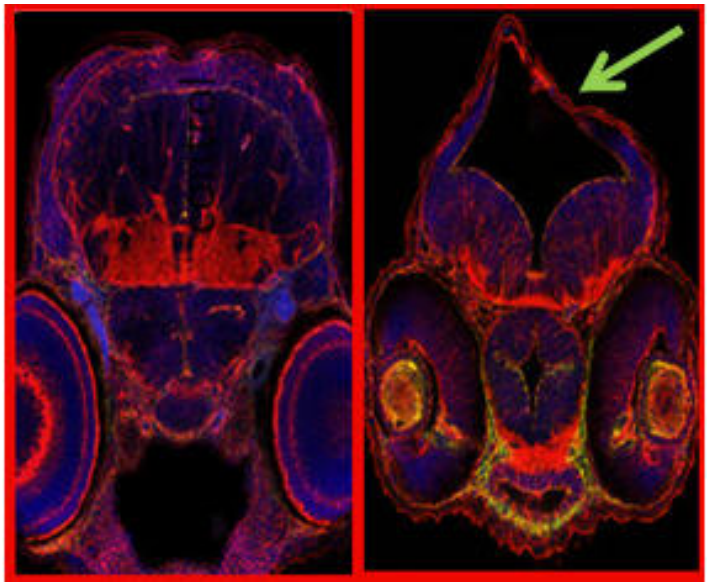
The zebrafish, a small fish found in most pet stores, is a powerful tool that allows scientists to study how genes affect the formation of organs during early development. As vertebrates, zebrafish have similar genes to humans. About 70% of human genes can be studied in zebrafish. In addition, zebrafish embryos are completely transparent, which makes organ development easy to see. Like humans, zebrafish have a neural tube which becomes the brain and spinal cord. To study the effect of specific genes, we disrupt or change a gene in zebrafish embryos, and then observe the developing zebrafish. If the zebrafish develop neural tube defects, we can be much more confident that problems with that gene are responsible for risk for neural tube defects in humans.

Previous research in our lab implicated the gene INADL in the development of anencephaly. INADL is active in the human brain and is involved in regulation of tight junctions (physical connections between cells), which are important for neural tube closure. Participants with anencephaly are more likely to have DNA variants (changes) in INADL than individuals without neural



IN THIS ISSUE:

tube defects. However, the presence of these variants is not enough evidence to conclude that INADL contributes to the development of anencephaly. To gather further evidence, we disrupted the INADL gene in zebrafish embryos. The resulting zebrafish had neural tube defects and small head size (microcephaly). We are currently testing the variants identified in participants with anencephaly to see if those specific changes in INADL will result in the same zebrafish cranial defects.



Left: normal zebrafish head
Right: zebrafish head with neural tube defect and microcephaly

Genetics Terms Defined

Cell - The basic building block of all living things. The human body is composed of trillions of cells.

DNA - DNA is short for deoxyribonucleic acid. DNA is a genetic code made up of a four-letter alphabet (each letter called a DNA base, or nucleotide) commonly denoted by the letters A, C, T, and G.

Gene - Genes are made up of DNA. Genes are segments of DNA that serve as the instructions for your body. A gene contains information that determines in part the traits, such as eye color, height, or disease risk, that are passed on from parent to child. We have two copies of most of our genes, one copy inherited from each parent.

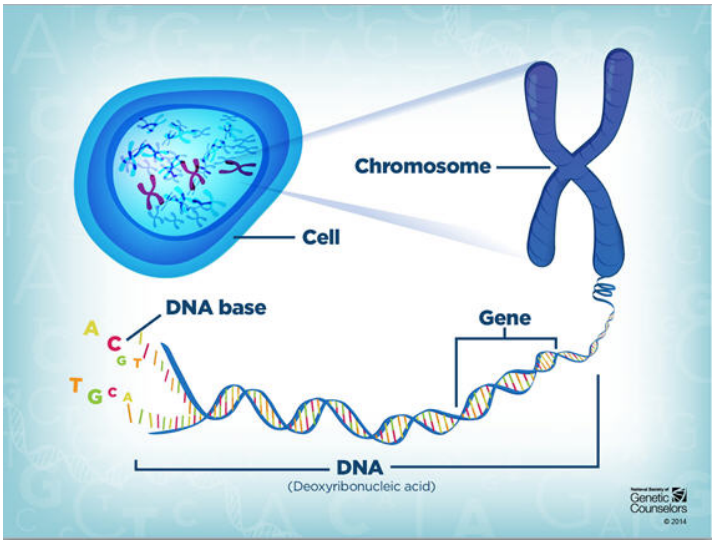
Chromosome - A chromosome is a tightly wound string of DNA containing hundreds to thousands of genes. Most of the cells in a human body contain 23 pairs of chromosomes (for a total of 46 chromosomes). For each pair, one chromosome comes from each parent. One pair, called the sex chromosomes, determines if we are male or female. Males have one X and one Y chromosome and females have two X chromosomes.

Genome - The collection of all DNA found in a human (3.2 billion letters of DNA) is called the genome.

Exome - Within the genome are about 30,000 genes. These genes account for only about 2% of the genome. This 2% - the collection of all genes - is called the exome.

Exome sequencing – The spelling out and reading of a person's DNA within the exome.

DNA variant - Sometimes called a mutation, a DNA variant is a change or difference in the DNA in comparison to most people.



Exome Sequencing Project Expands

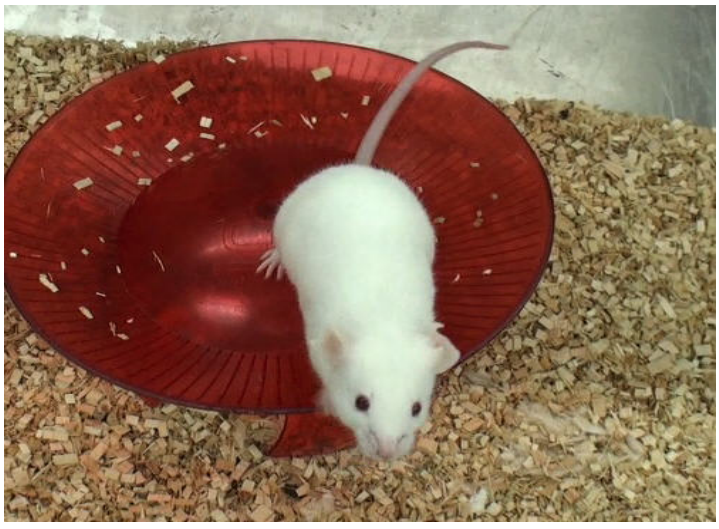
We all have DNA changes (variants) within our genes that make us unique from other people. Most DNA changes are not harmful but some DNA changes can cause medical conditions. Changes within genes are thought to contribute to neural tube defects. Exome sequencing is a technology that allows us to read or sequence all the genes in a person at one time. When exome sequencing was first developed it was very expensive, which limited the number of participants we could sequence. Our efforts began by sequencing two families; one with several individuals with spina bifida and another with several pregnancies with anencephaly.

Since that time, the cost of exome sequencing has dropped significantly, allowing us to expand the number of families sequenced. Through this method, we hope to identify changes in genes that are found more often in participants with neural tube defects than in their unaffected family members or the general population. Each participant sequenced so far has many DNA changes that could potentially contribute to neural tube defects. However, the DNA changes we are finding are different from participant to participant with very little overlap. This suggests that changes within many different genes, alone or in combination, may cause neural tube defects. As more is understood about the causes of neural tube defects, it is apparent that many different genetic and environmental factors may play a role, with the specific factors potentially varying from family to family. In short, we are finding that the genetics of neural tube defects are quite complex.

Krupp D. et al (2014) Missing genetic risk in neural tube defects: Can exome sequencing yield an insight? *Birth Defects Research (Part A)*, 100: 642-646.

Collaborative Research into CECR2 Highlights the Complexity of Neural Tube Defects

DNA changes (variants) in CECR2 can cause exencephaly in mice (equivalent to anencephaly in humans). However, not all mice with changes in CECR2 develop exencephaly. About half the mice of one type develop exencephaly but none of the mice of a different type develop exencephaly. Therefore, there must be other factors, in addition to CECR2, contributing to exencephaly in the affected mice.



Through genetic comparison of the two types of mice, Dr. Heather McDermid's laboratory at the University of Alberta, Canada identified 24 additional genes that may contribute to exencephaly in combination with genetic variants in CECR2.

Since mice and humans have fairly similar genes, a former graduate student in Dr. McDermid's laboratory, Renee Leduc, came to Duke to examine CECR2 and the 24 other genes in our participants with anencephaly and acrania. Seventeen participants (11% of those tested) were found to have a change in CECR2 that is thought to cause the gene not to work correctly. Changes were also identified in 17 of the other 24 genes. Of particular interest were changes in the genes DNMBP and TJP2, which like INADL described above, are also involved in tight junctions between cells. This research is consistent with the idea that multiple genetic factors in combination contribute to anencephaly and other neural tube defects.

85 Hours and 3 Minutes: The Participants' Point of View

By Harry Hatcher

Ten weeks into our pregnancy, my wife, Kristen, and I learned that our baby had a fatal abnormality called acrania. Following this devastating news, we began to search for answers and learned about a study at Duke University researching the causes of acrania and other neural tube defects. We were so thrilled to learn that somebody out there was doing something to learn more. It also happened to be college basketball season. I immediately became a Duke Blue Devil! I watched as many games on TV as I could. I even cried during games. It wasn't about a game or a sport or a player. It was about our son, Truett, and a university that cares enough to make a difference. As a sports enthusiast it was a special season for this dad.

Our C-section to meet our son was scheduled for April 7, 2015. Ironically, Duke played Wisconsin for the national championship on April 6th. As nervous and anxious as I was about the next day, I had to stay up and watch the game. I wasn't just cheering for Duke, I was cheering for Truett and for all the other families walking the same path. If you're a Blue Devil you know that Duke won that night. When the final buzzer sounded I hit my knees in the living room and was crying uncontrollably! Because Duke won, I felt like Truett won. I knew tomorrow was going to be a good day.

Truett was born at 10:55 am on April 7, 2015. Truett's cord blood was collected and sent to Duke University. He passed away in Kristen's arms at our home on April 10,

2015. He lived an amazing 85 hours and 3 minutes! It gives us hope and pride knowing that our son will make a difference in understanding acrania/anencephaly and to hopefully prevent this from happening to other families.

A few months later we got to meet some of the wonderful staff and researchers that conduct this study. It was an honor to meet the men and women who care about the families that are faced with neural tube defects. One of the highlights for me, as Truett's father, was meeting Casey Speer. Casey is the daughter of the late, Dr. Marcy Speer, who founded the Duke study. We know one day there will be answers, thanks to Dr. Speer and the researchers who continue what she started.

Anencephaly Psychological Impact

We conducted a project to examine the relationship between pregnancy management choices and psychological distress following the loss of a baby to anencephaly. Men and women who previously lost a baby to anencephaly completed questionnaires to collect information about their pregnancy and to assess levels of grief, post-traumatic stress and depression.

Individual experiences varied greatly but several patterns were observed:

- Women experienced more psychological distress than men.
- Psychological distress tended to decrease over time.
- Increased participation in organizational religious activities, such as attending church, resulted in less grief in both women and men.
- Pregnancy continuation resulted in less despair, avoidance and depression in women (this was not true for men).
- Pregnancy termination early in pregnancy resulted in less psychological distress than pregnancy termination later in pregnancy, especially for men.

Ultimately, coping with the loss of a baby is deeply personal and will be experienced differently by each person. We recommend health care providers discuss the risks and benefits of all options, but allow the family to make decisions that are best for them.

Cope H. et al (2015) Pregnancy continuation and organizational religious activity following prenatal diagnosis of a lethal fetal defect are associated with improved psychological outcome. *Prenatal Diagnosis*, 35: 761-768.

Fumonisin Likely Contributes to NTDs in Latin America

Fumonisin is a toxin produced by a mold that grows on corn in warm, relatively dry climates. It has been shown to cause neural tube defects (NTDs) in mice, and is thought to contribute to the higher numbers of babies born with NTDs in Latin America, where corn in 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.

Sphingolipids are molecules made by the body that protect cells against harmful environmental factors. Several genes are needed for proper production of sphingolipids. If changes within genes impair sphingolipid production, the body can be more susceptible to damage by fumonisin. In samples collected from Guatemalan women, we found a strong relationship between the number of corn tortillas eaten, the amount of fumonisin in the urine and the presence of sphingolipids in the blood. Currently, our team is looking for DNA changes in genes necessary for sphingolipid production that may make certain women more susceptible to fumonisin and, therefore, increase the risk of neural tube defects in their offspring.

Duke NTD Study Team

Front row left to right: Karen Soldano (lab research analyst), Rebecca Chen (student), Heidi Cope (study coordinator); Second row: Melanie Garrett (biostatistician), Jason Willer (lab research analyst), Aintzane Urbizu Serrano (postdoctoral associate), Matthew Pendleton (student); Back row: Tahir Khan (postdoctoral associate), Allison Ashley-Koch (principal investigator), Erica Davis (investigator), Zac Kupchinsky (zebrafish manager)

Enrollment Update

Due to funding limitations, study enrollment has been limited to anencephaly trios (baby with anencephaly plus both parents) for the last few years. In 2014 and 2015, 58 additional trios joined the study. In total, we have collected DNA samples from 5,711 individuals in 1,502 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!

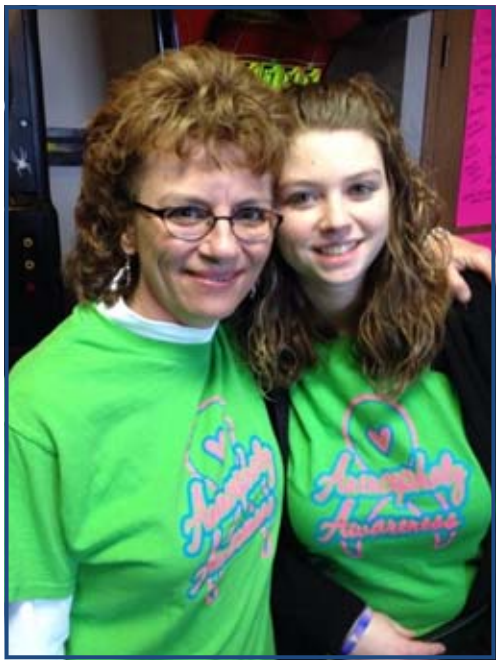
In Loving Memory

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.

In Honor of Hallie

By Alyssa Branowitzer

While pregnant with our second child, my husband and I found out she had anencephaly. When we received the diagnosis we had never heard of anencephaly before. In honor of our daughter, Hallie, we decided to fundraise to benefit anencephaly research and to increase awareness of anencephaly in our community. This was very important to us in many ways. Knowing that we could not change our situation, we saw it as an opportunity to help prevent future families from experiencing a similar loss. Our family and friends helped us to talk with local businesses and collect donations. It was great to see the community come together in honor of Hallie. As time passes, our love for our daughter never fades. We continue to raise funds in different ways so that our daughter's life can live on by helping others.



Alyssa and her mother sporting anencephaly awareness t-shirts designed for their event.

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 team has been awarded research funds or grants from March of Dimes, National Institute of Health (NIH) 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!

To make a financial gift to the 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 Duke University Medical Center, NTD Research Fund, 300 N Duke Street, Durham, NC 27701.