DUKE center for HUMAN GENETICS

News About...
NEURAL TUBE DEFECT Research

Volume 6, Summer 2006

Welcome from Dr. Marcy Speer

On behalf of the entire NTD Research Team here at Duke, I'd like to welcome you to this latest edition of "News About…Neural Tube Defects Research." This newsletter is for the families who so generously contribute their time to support our research into Neural Tube Defects and the genes that may influence NTDs. I hope that you enjoy reading about our latest work. We have been making great progress in our genomic screen follow-up as well as continuing to investigate candidate genes for NTDs. None of this work would be possible without your help. Thank you!

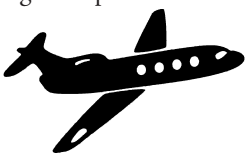
General study enrollment update

We have collected DNA samples from 4080 individuals in 1097 families. Over the past year we have sampled 571 individuals. Thank you to anyone who contributed a sample during the past year, or since 1993 when the study began.

We have received some inquiries as to the breakdown of families enrolled in the study. The majority (381) of our families are Caucasian families in which one family member has a lumbo-sacral myelomeningocele. The next largest group of families (310) has two or more family members diagnosed with one of the many types of NTDs. These families represent all racial and ethnic categories. For the remaining families that are Caucasian and have only one family member with an NTD, there are 49 with thoracic level myelomeningoceles, 86 with lipomyelomeningoceles, and 66 with other NTD diagnoses. We have 53 non-Caucasian families enrolled in which only one family member has an NTD. The remaining families (111) have family members who have been diagnosed with anencephaly, encephalocele, craniorachischisis, Meckel-Gruber Syndrome, or other similar NTDs.

Visits from NTD Study Personnel

In many cases we are able to visit families in person to complete their enrollment in the NTD study. During visits, we answer any questions participants have about our study, draw blood samples, and complete any other paperwork. We also briefly look at the lower back of those family members who do not have an NTD to look for signs of spina bifida occulta. Since January 2005, NTD Staff members have visited families in 20 states throughout the United States. We enjoyed meeting each and every family and really appreciate your participation in the Neural Tube Defect research study.



Alabama	Michigan	Pennsylvania
Colorado	Missouri	South Carolina
Florida	New Jersey	Texas
Georgia	New York	Utah
Indiana	North Carolina	Virginia
Louisiana	Ohio	Wisconsin
Massachusettes	Oregon	

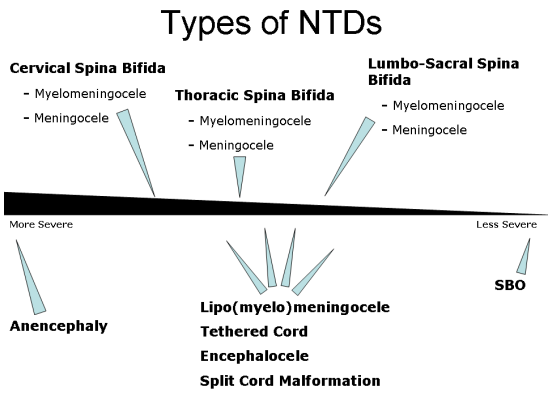
We will continue to travel to 2-3 areas each month and can usually accommodate your scheduling needs, including evening or weekend visits. Please contact us if you would like to schedule a home visit to complete your family's enrollment in the study. We are in the process of planning trips to Alabama, Florida, California, Indiana, Pennsylvania, Utah and Arizona. If you live in one of these areas, we look forward to speaking with you!

NTD Spectrum

Neural tube defect (NTD) is an umbrella term, under which several different conditions fall. All of these conditions involve the improper or incomplete closure of the neural tube somewhere between the third and fourth weeks of embryological development. If the portion of the neural tube that will form the brain/skull does not close correctly, anencephaly, acrania, or encephalocele can develop. Anencephaly and acrania fall on the most severe end of the NTD spectrum, as these conditions are not compatible with life. Open neural tube defects, which occur along the spine (myelomeningocele), can range from a less severe NTD to a more severe NTD depending on the size of the opening as well as the location along the spine where the opening occurs. The higher up the spine the opening occurs the more nerves that may be damaged. Closed neural tube defects (lipomyelomeningocele, tethered cord, split cord malformation, etc) can again vary from less severe to more severe depending on the location of the spine where the defect occurs and the size of the defect (a larger defect can damage more nerves than a smaller defect).

The medical records and radiographs which we collect as part of your participation in our study allow us to accurately categorize your family by the type and level of NTD in the family. We do this in the event different genetic and environmental factors play a role in each different type of NTD, and possibly even in each level of the same type of NTD. For example, it is possible that a lipomyelomeningocele may be caused by different factors than anencephaly and even different factors than myelomeningocele, or that a thoracic level myelomeningocele is caused by different

factors than a lumbo-sacral myelomeningocele. On the other hand, it may be true that these conditions share the same underlying genetic predisposition, with different "triggers" leading to different forms of NTDs, even within the same family!



How Common are Neural Tube Defects?

Approximately 1 in 1,000 babies born in the United States has a neural tube defect, which makes NTDs among the most common and serious birth defects. The Center for Disease Control and World Health Organization estimate that at least 3,000 pregnancies in the US and 400,000 in the world are affected by spina bifida or anencephaly each year. The incidence (how many new cases occur each year) and prevalence (number of currently diagnosed individuals) appears be affected by many complex factors that still require much research. Two important factors appear to be geographical location and ethnic and/or racial background.

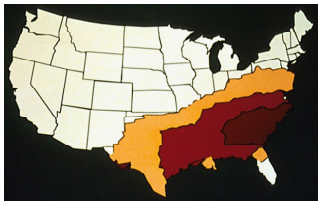
Ethnic/Racial Background

A person's race and/or ethnic background appear to also affect the prevalence of NTDs. Again, not all races or ethnic backgrounds have been adequately studied, but based on current information the prevalence appears to reflect a higher incidence among certain populations. In the United States the ratio appears as follows:
Non-Hispanic Caucasian < Black and Asian < Hispanic

Non-Hispanic Caucasian < Black and Asian < Hispanic
Prevalence of NTDs

(Prevalence increases from left to right)

U.S. Incidence



>1 per 2,000 live births in the US

Geographical Location

Birth rates of NTDs appear to be highest in certain "hot spots" around the world, including Northern China, India, British Isles, Latin America and the Southeastern portion of the United States, particularly the Appalachian Mountain region. The number of pregnancies and family affected by NTDs around the world is still being studied and there may be other regions with high birth rates also. Below is a map of the United States, which shows a well-documented "gradient affect", with higher prevalence occurring in the eastern section of the country.

Duke Collaboration with Guatemala and the USDA

The Center for Human Genetics has embarked on a collaborative research effort in 2005 with researchers and physicians in Guatemala and scientists at the United States Department of Agriculture. Guatemala and other Latin American countries have some of the highest birth rates on NTDs (up to 1 in 100 births) in the world. We are working together to determine what genetic and environmental factor potentially cause this high rate, and whether or not our countries share some of these factors. One hypothesis is that there are genes that interact with a common environment exposure in Guatemala, called fumonisin. Fumonisin is a mycotoxin that frequently contaminates maize (corn) in some areas of the world. Eating contaminated corn appears to affect the birth defect rate. While this evidence does not suggest Americans should reduce their intake of corn products found in the US, it does raise questions about the gene-environment interaction in Guatemala and therefore, may help detect some causes of NTDs. We are very excited about domestic and international collaborations that help move us all towards our common goal of solving this complication puzzle.

Lab update

Recent studies have identified candidate genes for spina bifida (SB) related to DNA repair (Olshan et al. 2005) and mitochondrial uncoupling proteins (Volcik et al. 2003). Promising associations in the candidate genes APEX1, ERCC2, and UCP2 have therefore been selected for follow up utilizing the NTD Collaborative Study lumbosacral myelomeningocele pedigrees.

The Mitochondrial Uncoupling Protein UCP2

The uncoupling proteins (UCPs) are mitochondrial inner-membrane proteins that have been hypothesized to be involved in the regulation of weight gain through a variety of direct and indirect methods. Variations in UCP2 have also been shown to associate with an increased risk of SB (Volcik et al. 2003). Both maternal obesity and diabetes have been associated with a higher risk of neural tube defects (NTDs) (Watkins et al. 2003; Shaw et al. 2000; Loeken 2005). Thus, the potential roles of UCP2 in influencing energy expenditure and insulin secretion make it an interesting candidate gene for NTDs (Walder et al. 1998; Cassell et al. 1999; Nagy et al. 2004; Rousset et al. 2004; Krauss et al. 2005). Studies in human populations have not found any strong linkage of UCP2 to either obesity or Type II diabetes, though multiple studies have detected associations with modest effects, often in smaller subpopulations. Thus, though no one specific role for UCP2 in the risk for either obesity or diabetes has been established, the

large number of related associations is interesting nonetheless. Neither the single nucleotide polymorphism (SNP) tested in the Volcik et al. paper nor the insertion/deletion fragment showed association with SB in the NTD Collaborative Study lumbosacral myelomeningocele pedigrees.

DNA Repair Genes APEX1 and ERCC2

APEX1 (AP endonuclease 1, also known as APE1), is a vital component of the base excision repair pathway, acting to cleave the 5' side of the abasic site and recruiting Pol β to the site. APEX1 has also been shown to play a role in other cellular functions, such as the redox regulation of transcription factors including p53. ERCC2 (excision repair cross-complementing rodent repair deficiency complementation group 2, also known as XPD), is an ATP-dependent DNA helicase involved in nucleotide excision repair. ERCC2 is also a vital component of the TFIIH basal transcription factor. Defects in ERCC2 have been shown to cause xeroderma pigmentosum group D (XP-D), xeroderma pigmentosum group D combined with Cockayne's syndrome (XP-D/CS), trichothiodystrophy (TTD), and cerebro-oculo-facio-skeletal (COFS) syndrome. After testing 7 SNPs (1 SNP in APEX1 and 6 SNPs in ERCC2), the most significant finding, so far, is in an intronic SNP of ERCC2, with a p-value of 0.04. None of the other six markers tested, to date, have shown any evidence of association with SB (p-value < 0.1). Further work will thus investigate linkage disequilibrium in the region of the ERCC2 intronic SNP.

Candidate Genes Update: Folate Pathway Genes

Dietary folate supplementation plays a recognized role in the prevention of NTDs, but the mechanism by which it acts is poorly understood. We are studying eleven genes in the folate pathway that will help us clarify the relationships between folate, homocysteine, and neural tube defects. FOLR1, FOLR2, and SLC19A1 bind folate and transport it into the cell. Vitamin B12 is also necessary for proper functioning of the folate pathway and it is brought into the cell by TCN2. MTHFD1 and SHMT convert between different forms of folate within the cycle. MTHFR is the most widely studied NTD candidate gene and is also an important member of the folate pathway.

Part of the folate cycle, homocysteine is an amino acid which can accumulate due to low dietary folate, low vitamin B12, and/or genetic factors, and has been observed at mildly elevated levels in some NTD mothers. The conversion of homocysteine to methionine links the folate pathway to a methylation pathway and is thought to be a key step in reducing homocysteine levels. Two enzymes perform this conversion: BHMT and MTR. We also studied the gene that maintains MTR in its active state, MTRR. Alternatively, homocysteine can be degraded into cystathionine by another gene – CBS.

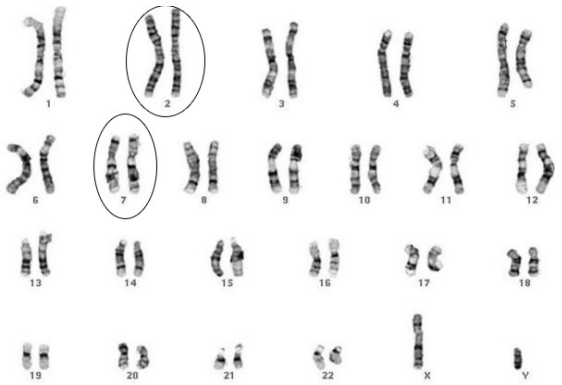
All of these genes have been previously studied as candidate genes for NTDs by the Duke study or other researchers. We hope by studying these genes and environmental risk factors, such as preconception folate supplementation, we can elucidate the complex relationships between the genes, folate, and NTDs.

Boyles AL, Billups AV, Deak KL, Siegel DG, Mehltretter L, Slifer SH, Bassuk AG, Kessler JA, Reed MC, Nijhout HF, George TM, Enterline DS, Gilbert JG, Speer MC, and the NTD Collaborative Group. (2006) ***Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene-environment interactions****. Environmental Health Perspectives. (submitted)*

Genomic Screen update

Last year, the genome screen for Neural Tube Defects was completed (Rampersaud et al. 2005). This screen included 44 multiplex (more than one affected individual in the family) and over 400 polymorphic microsatellite markers were genotyped on these families to cover the entire genome. The genomic screen used linkage analysis techniques, which evaluates for co-segregation of disease (NTD) with the genetic marker(s). Using this method, regions of linkage on chromosomes 7 and 10 were identified. Further investigations of the genomic screen data identified a single large family as primarily driving the linkage results on chromosome 7.

We followed up the microsatellite screen with a high-density whole genome single-nucleotide-polymorphism (SNP) screen to confirm previous findings and to establish regions of linkage on the large NTD pedigree responsible for linkage on chromosome 7. The



regions identified mapped close to the telomeres of 2q and 7p. Since, there is a lot of cross over events (recombination) in the telomeres and known that 2-16% of non-syndromic NTDs are associated with cytogenetic abnormalities, we plan to perform cytogenetic studies on this family to determine if there is a deletion or translocation near the telomeres of chromosomes 2 and 7 that may be responsible for the development of the NTD. Several promising candidate genes reside in the 2q and 7p regions of interest, including FZD7, CASP8/10, PAX3,

MEOX2, TWIST1, and HOX genes. We are also in the process of finemapping these linked areas, which involves genotyping additional genetic markers to narrow the chromosomal locations we follow up for candidate gene studies.

Such large families, with multiple affected individuals and several participating family members, provide significant information for genetic analysis of neural tube defects. These extensive NTD pedigrees are an important resource for narrowing the search for NTD candidate genes.

Rampersaud E, Bassuk AG, Enterline DS, M T, Siegel DG, Melvin EC, Aben J et al (2005) Whole genome-wide linkage screen for Neural Tube Defects reveals regions of interest on chromosomes 7 and 10. Journal of Medical Genetics.

Illumina update

We are also following up the whole genome screen with an Illumina platform that will genotype over 5000 single-nucleotide polymorphisms (SNPs). Approximately 45-50 families will be included in this screen. This SNP screen provides a much higher density of marker coverage throughout the genome. We hope the information extracted will confirm the regions identified on chromosomes 7 and 10, and narrow the candidate gene interval.

Family Update

Please keep us in mind when important changes happen in your family. We like to ensure that all of our family trees and mailing lists are accurate. We would like to know if there are any major changes in the health status of family members, such as someone diagnosed with a new medical disorder, if another family member is diagnosed with a neural tube defect or if there are new members of your family. Also, if you should move, please give us your updated contact information. You may give us your updates by calling us toll-free at 866-DUKE-NTD, directly at 919-684-0767, or by sending an email to ntd@chg.duhs.duke.edu. Thanks!

In Loving Memory

We would like to express our sincere condolences to families who have lost a pregnancy 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.

Kids Korner

Unscramble the words:

1.) B N A
I R

2.) E S N
P I

3.) A H
D E

4.) U L R
E N A

5.) K A
B C

6.) L E C R A
H H I W E

7.) H E C R
A E R S

8.) N T I S
E G E C

ANSWERS: 1–brain 2-spine 3-head 4-neural 5-back 6-wheelchair 7-research 8-genetics

Who Pays for the Research?

Conducting the genetic research studies that create neural tube defect breakthroughs is painstaking and expensive work that relies on funding support from both public and private sources. Substantial funding is typically only granted to researchers with strong research plans and programs already in place. With the participation and support of the more than 1100 families already enrolled in the study, you have helped us to develop one of the strongest NTD genetic studies in the nation. Since 1993, when the Hereditary Basis of Neural Tube Defects study began, the Duke CHG 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, and from families that are enrolled in the study or whose lives have been touched by neural tube defects.

On occasion, we are asked if we can accept donations to support the NTD research, sometimes in memory of a baby or loved one who has passed away. If you or someone you know would like to make a gift, the Center for Human Genetics has created the Duke CHG Neural Tube Defect Research Fund. To make a financial gift to neural tube defect research, you may visit the secure web site of the gift records office at www.giftrecords.duke.edu and click on "Make a Credit Card Gift Online," or you can send your tax-deductible donation to the address below:

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