Duke University Medical Center

News About...

Chiari Type I Malformation Research

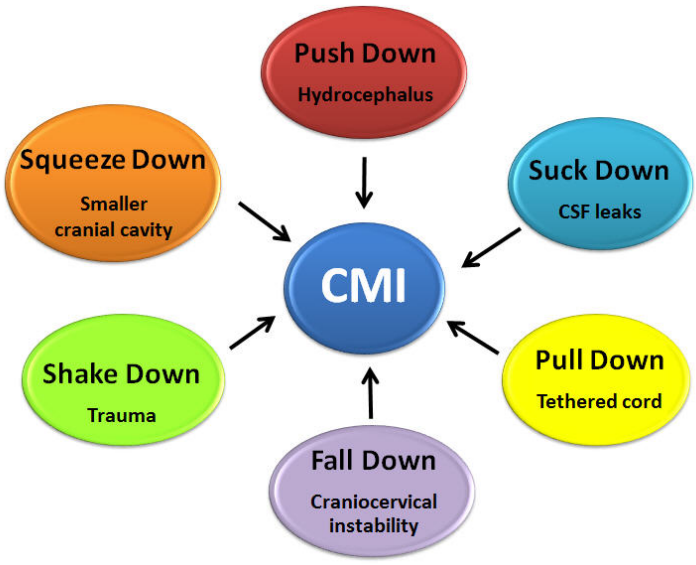
Spring 2014

The Search for Chiari Genes Continues

Chiari Whole Genome Linkage Screen Demonstrates the Importance of Clinical Subtyping

Chiari Type 1 Malformation (CM1) is a highly variable condition. Individuals with CM1 may have vast differences from one another including differing symptoms, response to surgery, and age of onset. Many researchers believe that CM1 is a condition consisting of several subtypes. Each subtype may be caused by different genetic and/or environmental factors.

We previously completed a whole genome linkage screen of 367 individuals in 66 CM1 multiplex families (families with two or more members diagnosed with CM1). Whole genome linkage analysis was performed in an effort to locate specific regions of the genome that may contain genes that cause or contribute to CM1. When all families were analyzed together, little evidence was found for a common genetic cause. Therefore, families included in the screen were divided into two subtypes; families with classical CM1 and families with symptoms of connective tissue disorders. Classical CM1, "squeeze down" in the figure at right, is thought to result from a too small posterior fossa (space in the skull which holds the brain stem and cerebellum). In contrast, individuals with connective tissue disorders, "fall down" in the figure below, are thought to acquire CM1 as a result of craniocervical instability (an unstable joint between the cervical spine and head). Joint flexibility or instability is a common symptom of connective tissue disorders.



Adapted from Milorat et al. Acta Neurochir (2010) 152:1117–1127

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Dividing families into these clinical subtypes greatly improved the results, resulting in identification of several different regions of the genome that may contain genes important in the development of CM1. Different regions of the genome were implicated for each clinical subtype. Regions on chromosome 8, 9, 12 and 17 were found for families with classical CM1. Regions on chromosome 1, 7, 9 and 18 were found for families with symptoms of connective tissue disorders. These findings were published in the scientific journal PLoS ONE (the complete reference is below).

Following the success of this subtyping method, a second approach was used to group families. Brain MRIs were used to collect detailed measurements of the posterior fossa (PF) region. This allowed families with similar PF measurements to be analyzed together. The most significant finding from this analysis was a region on chromosome 22 in families with large PF height. These results were published in the scientific journal Annals of Human Genetics and were featured on the cover of that issue (the complete reference is below).

Markunas C, Soldano K, Dunlap K, Cope H, Asiimwe E, et al. (2013) Stratified Whole Genome Linkage Analysis of Chiari Type I Malformation Implicates Known Klippel-Feil Syndrome Genes as Putative Disease Dandidates. PLoS ONE 8(4): e61521.

Markunas C, Enterline D, Dunlap K, Soldano K, Cope H, et al. (2014) Genetic Evaluation and Application of Posterior Cranial Fossa Traits as Endophenotypes for Chiari Type I Malformation. Annals of Human Genetics 78:1-12.

The Genome Screen is Complete – Now What?

The genome screen described above revealed several broad regions of DNA that may contain genes that cause or contribute to CM1. The next step is to study genes within these regions to identify specific DNA changes that increase the chance of a person having CM1. In particular, we are interested in identifying "rare" changes that occur very infrequently in the world population. Because they occur so infrequently, rare changes that are present only in individuals with CM1 or more frequently in individuals with CM1 are more likely to play a role in the development of the condition.

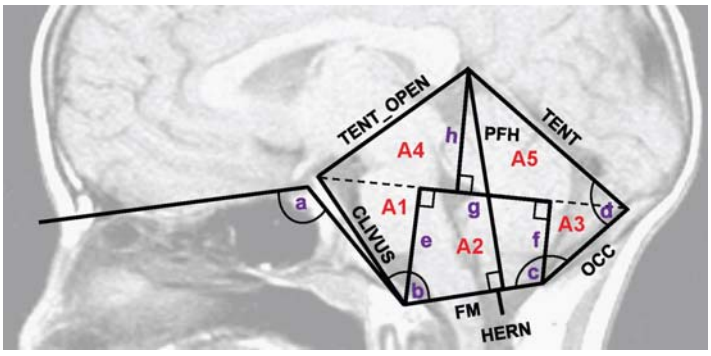
Within the regions identified for families with classical CM1 are two genes (GDF3 on chromosome 12 and GDF6 on chromosome 8) known to cause Klippel-Feil syndrome (KFS), a condition characterized by fusion of any two of the seven cervical vertebrae. Since 3-5% of individuals with CM1 are diagnosed with KFS, we choose to look at these genes first. GDF3 and GDF6 were sequenced (read) in individuals with CM1 from families that were included in the genome screen. No important DNA changes were found in GDF3. However, several interesting DNA changes, including one previously found in individuals with KFS, were found in GDF6. While this is promising preliminary research, further research needs to be completed to confirm the involvement of GDF6 in CM1.

We plan to sequence both GDF3 and GDF6 in a larger group of individuals with CM1.

In addition to looking at the two GDF genes, we also used Illumina MiSeq, a benchtop next generation sequencing instrument capable of sequencing many genes for multiple people at one time (pictured at the bottom of page 2), to sequence 28 additional genes within our regions of interest in 94 individuals with CM1. A preliminary analysis has identified a small number of changes within these genes in individuals diagnosed with CM1 that have not been seen before. Future research will focus on sequencing additional genes within our regions of interest in a larger group of individuals with CM1 (see our new collaborators on page 5).

Studies of Brain MRIs Show Criteria Used to Diagnose CM1 Can Be Improved

While no standardized criteria exist to diagnose CM1, individuals are usually considered to have CM1 if one cerebellar tonsil is herniated 5 mm or more or both tonsils are herniated 3 mm or more. In an effort to characterize brain MRI findings in CM1 multiplex families, detailed measurements of the posterior fossa (PF) region were taken on brain MRIs obtained from 92 individuals with CM1 and 28 unaffected relatives from 50 families.



Several PF measurements were shown to be heritable, meaning related individuals tend to have similar measurements. PF height (PFH) was found to be the most heritable trait and also the trait that best correlated with the diagnosis of CM1. Importantly, tonsillar herniation, which is the gold standard by which individuals are diagnosed, was *not* found to be heritable. This provides further evidence that tonsillar herniation likely occurs secondarily to a small PF. It is well known that tonsillar herniation does not correlate well with symptoms, may not be necessary to cause disease (Chiari 0) and therefore, may not be the best criterion to use for diagnosis.

Dura Expression Study Identifies Chiari Subtypes

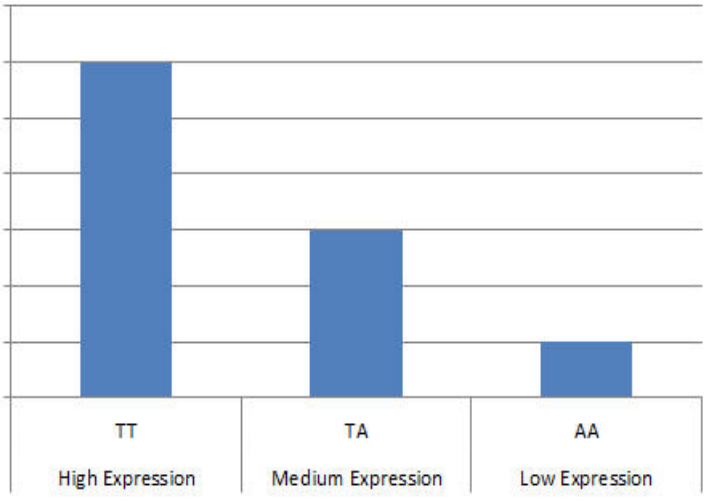
In 2010, Conquer Chiari, a non-profit organization dedicated to improving the experiences and outcomes of patients with Chiari and syringomyelia through education, awareness and research, awarded the Duke Chiari team a grant to further study the genetics of CM1. This funding was used towards research to identify subtypes of CM1 by looking at gene expression patterns (genes turned on and off) in samples of blood and dura (the outmost layer covering the brain) from CM1 patients. Samples were collected by two neurosurgeons at Duke, Drs. Herbert Fuchs and Gerry Grant (now at Stanford), from 70 patients between the ages of 10 months and 17 years undergoing decompression surgery for CM1. Clinical information consisting of medical records, brain MRIs and symptoms was also collected in hopes of matching gene expression patterns to clinical characteristics. The brain MRIs were used to take detailed measurements of the

posterior fossa (PF), the small space in the skull which holds the brain stem and cerebellum.

Of the 70 patients enrolled in the study, complete data were obtained for 44. Data from these 44 patients were analyzed to determine CM1 subtypes. In total, four different CM1 subtypes were identified. Patients with these subtypes had different patterns of genes turned on and off and also different PF measurements. Children in one subtype of particular interest had older fathers. While this was a small study, it confirms that CM1 is a highly variable condition with potentially several underlying subtypes. Further study of CM1 subtypes is needed.

Linking Data Together Provides Further Clues

We are in the process of conducting additional analyses using the data generated from the dura expression study (described on page 3). Gene expression patterns generated as part of the study are now being integrated with participant *genotypes*. Gene expression describes how often a gene is used by the body. On the other hand, a person's genotype is their genetic code, or DNA, made up of a four letter alphabet (T, A, C and G). The genotype between two individuals may differ at any one position (TT vs. TA, for example). The main goal for integrating these two sources of information is to identify DNA changes that affect the expression of a gene, as these changes are likely to have direct impacts on body functions.



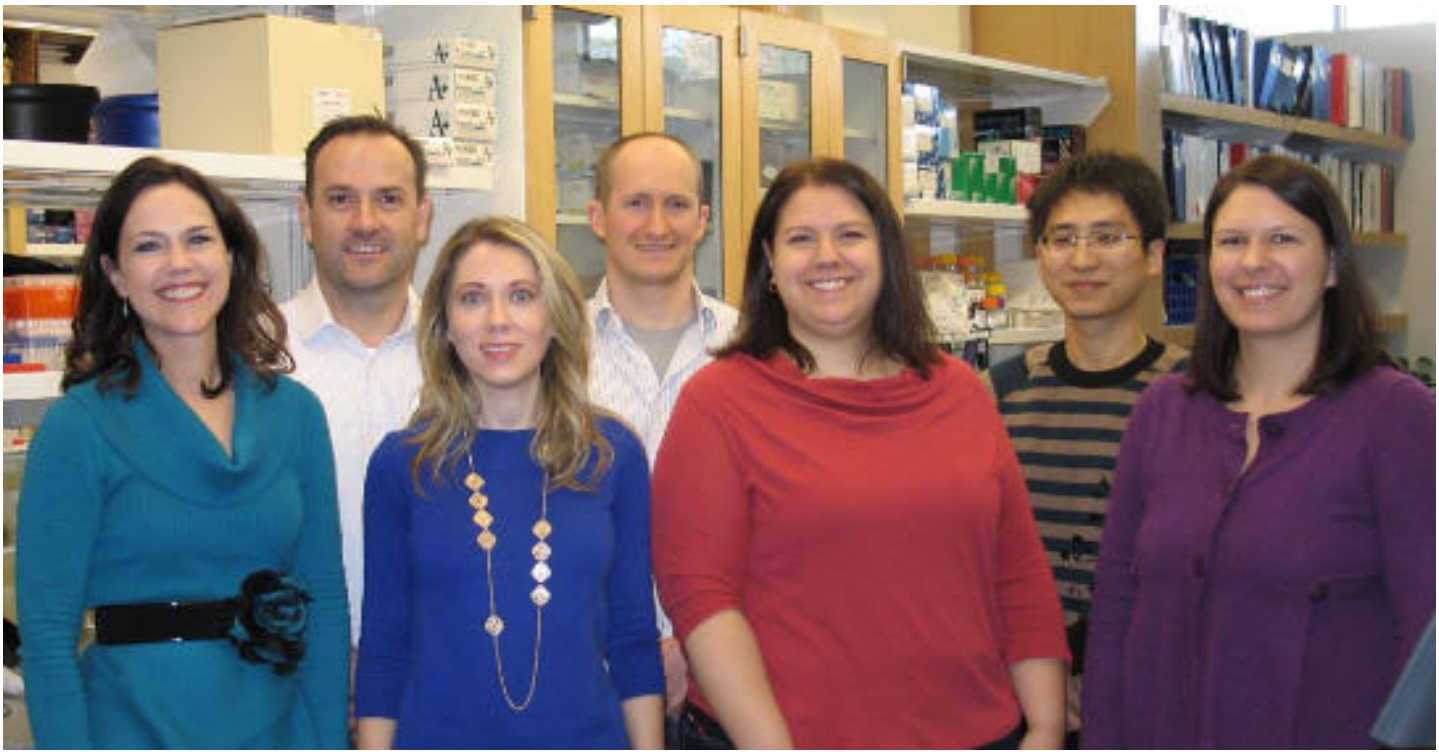
In the example at left individuals with genotype AA have much lower expression of a gene (gene is turned on less often) than individuals with genotype TT. If individuals with CM1 were more likely to have genotype AA, this could mean that low expression of that gene may cause or contribute to CM1. In a preliminary analysis we have identified hundreds of these associations, several of which occur much more frequently in individuals with CM1 than in other populations.

Chiari Study Enrollment Update

Additional families enroll in the study every year. To date, we have collected DNA samples from 1,616 individuals in 325 families from across the United States. Of these 325 families, 265 families (82%) are multiplex families, meaning there are two or more individuals diagnosed with CM1 in the family. The other 60 families are singletons, meaning there is only one individual diagnosed with CM1 in the family.

Thank you to anyone who contributed to the research since 1994 when the study began. Research toward a better understanding of the causes of CM1 would not be possible without your help. We continue to enroll additional families with two or more members diagnosed with CM1.

Duke Chiari Study Team

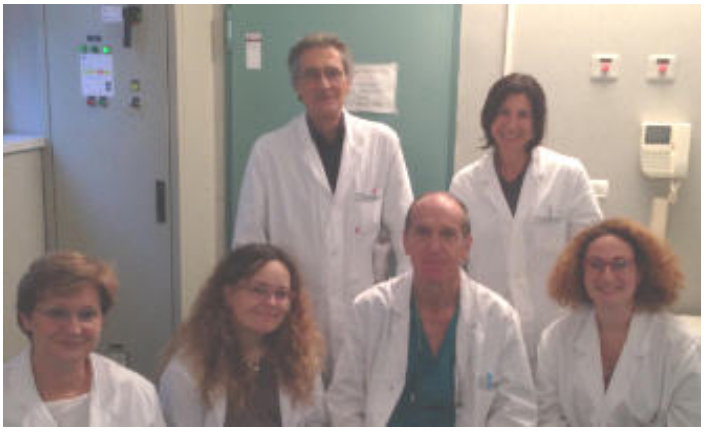


Left to right: Allison Ashley-Koch (Principal Investigator), Simon Gregory (Principal Investigator), Heidi Cope (Study Coordinator), Eric Lock (Postdoctoral Associate), Karen Soldano (Lab Research Analyst), Shifu Sha (Graduate Student), Melanie Garrett (Biostatistician)

Duke Chiari Study Forms New Collaborations

Dr. Shane Tubbs

As discussed earlier in this newsletter, we recently completed a CM1 whole genome linkage screen and identified evidence for linkage to genomic regions containing two genes, GDF3 and GDF6, which have been previously implicated in Klippel-Feil syndrome (KFS). In light of these findings, we have initiated collaboration with an experienced KFS researcher, Dr. Shane Tubbs, Director of Research in Pediatric Neurosurgery at the Children's Hospital of Alabama. Dr. Tubbs will collect saliva samples from patients diagnosed with both KFS and CMI so that we can look for DNA changes in the GDF3 and GDF6 genes in these patients.

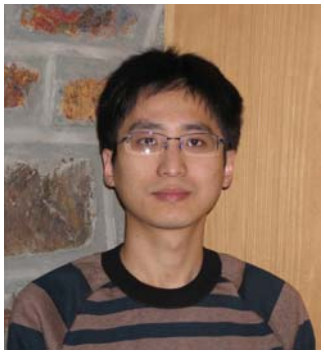


Dr. Palma Ciaramitaro (top right)

In order to increase the numbers of families available for genetic studies and to investigate CM1-associated genes in other

populations, we have initiated collaboration with a Chiari researcher in Italy, Dr. Palma Ciaramitaro at Centro Regionale Esperto Siringomielia-S. DNA samples and clinical information from CM1 multiplex families collected by Dr. Ciaramitaro will be sent to Duke for purposes of this study. Samples and data from the first eight families have already arrived!

Shifu Sha



Shifu Sha is a 3rd-year PhD candidate from Nanjing University Medical School in China whose doctoral research is primarily focused on the pathogeneses of Chiari malformation and syringomyelia as well as the surgical management of scoliosis (a spinal disease commonly associated with Chiari). Shifu has joined the Duke Chiari study team as a joint-training student for a period of 12 months to learn molecular genetic techniques.

Chiari Researchers Come Together

ASAP's Chiari and Syringomyelia Conference

In July 2013, the American Syringomyelia & Chiari Alliance Project (ASAP) held their annual Chiari and Syringomyelia Conference in Los Angeles, California. The meeting was aimed at raising awareness of Chiari and to provide individuals with Chiari and their families an update in the current knowledge of Chiari symptoms, treatments and research. The meeting was a tremendous success, both scientifically and socially. Dr. Eric Lock spoke about the genetic basis of Chiari malformations in the context of the human genome, sequence variation and genetic risk. Presentations were videotaped and will appear on the ASAP website soon, http://asap.org/index.php/get-involved/conference/.

Conquer Chiari Research Conference

In November 2012, Conquer Chiari in partnership with Column of Hope held their bi-annual research conference in Chicago, Illinois. Top Chiari researchers and neurosurgeons gathered to discuss their research. Many outstanding presentations were given over a two day period. Presentations covered a wide range of topics, including syrinx formation, quality of life, cognitive function, disease mechanisms, surgical outcome and techniques. The Duke Chiari research team, represented by Dr. Simon Gregory presented, "Identification of Pediatric Chiari Type I Malformation Subtypes Using Clinical and Biological Factors." It was a wonderful experience for all attendees and a good chance for researchers to share and discuss ideas. The conference booklet with summaries of the presentations can be found on the Conquer Chiari website, http://www.conquerchiari.org/ccresearch/conference.html.

CSF's "Think Tank" Meeting

In April 2012, the Chiari & Syringomyelia Foundation (CSF) had their annual "Think Tank" meeting in Miami, Florida. Members of the CSF Medical Research Board, Board of Directions, Board of Trustees and staff met to discuss current and future research strategies. The Duke Chiari research team, represented by CSF Scientific, Education and Advisory Board Executive Committee Chair, Dr. Allison Ashley-Koch and, then graduate student, Dr. Christina Markunas,

presented, "Genetic Dissection of Chiari." Dr. Markunas's presentation was recorded and can be viewed on the CSF website, http://csfinfo.org/event/csf-think-tank-meeting1/?eID=60.

Walking for a Good Cause

The Duke Chiari study team regularly participates in local Chiari fundraising and awareness events. Members of the team have taken part in the 2012 and 2013 Chiari & Syringomyelia Foundation (CSF) unite@night walk and the Conquer Chiari Walk Across America. These walks are a great opportunity for us to connect with local Chiari families and remind us why our research is so important.

Left to right: Christina Markunas, Simon Gregory, Karen Soldano, Allison Ashley-Koch, Heidi Cope

Screening Participants for Connective Tissue Disorders

As we continue to research the genetic causes of CM1, it has become increasingly clear that connective tissue disorders often co-occur with CM1 and may be important in distinguishing CM1 subtypes. In order to learn more about connective tissue disorder symptoms in study participants we recently mailed a measure called the Beighton to all enrolled study participants. The Beighton is used to measure joint hypermobility through a simple 9-item physical exam. Joint hypermobility is a common symptom of connective tissue disorders. Most people will score less than 2 points, while scores of 5 points or higher indicate the presence of joint hypermobility. We now have Beighton scores for 449 participants. Joint hypermobility was reported by a significant number of participants; 84 participants (19%) scored between 2 and 4 points and 67 participants (15%) scored 5 points or higher. Thank you to those participants who took the time to complete and return this helpful measure!

Goodbye to Dr. Markunas

Christina Markunas was a graduate student co-advised by Drs. Allison Ashley-Koch and Simon Gregory and an integral member of the Duke Chiari study team. During her time with us she conducted exemplary research which resulted in several publications and received the first Chiari Fellowship from the Chiari & Syringomyelia Foundation (CSF). Dr. Markunas completed her time with us upon receiving her Ph.D. in Genetics and Genomics from Duke University in May, 2013. She is currently a postdoctoral IRTA fellow working in the Reproductive Epidemiology Group at NIEHS under the mentorship of Dr. Allen Wilcox.

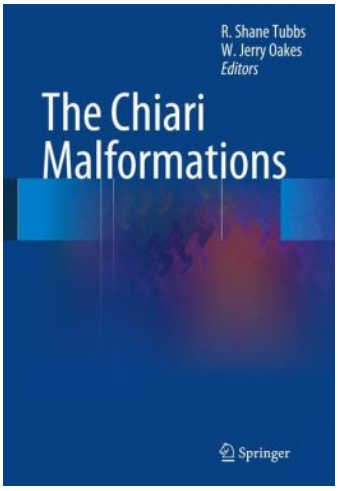
Keep Us Updated

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 Chiari. 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 toll-free at 877-825-1694 or by sending an email to chiari@chg.duhs.duke.edu. Thank you!

Chiari Book Published

The Chiari Malformations, a book published in 2013, was written by leading Chiari researchers and physicians with the purpose of detailing the medical community's current understanding of Chiari I and II malformations. Duke Chiari study



team members, Drs. Christina Markunas, Allison Ashley-Koch and Simon Gregory, contributed a chapter about the genetics of these conditions. The Chiari Malformations is now available for purchase through most major book sellers.

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For periodic updates on Chiari research being conducted at the Duke University Medical Center "like" us on Facebook at:
http://www.facebook.com/pages/Chiari-Type-1-Malformation-Study-at-Duke-University-Medical-Center/258936470795236

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 Chiari team has been awarded research funds or grants from the National Institute of Neurological Disorders and Stroke (NINDS), Conquer Chiari, the American Syringomyelia & Chiari Alliance Project (ASAP), the Chiari & Syringomyelia Foundation (CSF), and from private donors whose lives have been touched by Chiari malformation.

We are often asked if we can accept donations to support the Chiari research, sometimes in memory of a 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 Chiari Research Fund. To make a financial gift to Chiari 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 3912376**. Or you can mail your tax-deductible donation to the address below:

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Chiari Research Fund
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Durham, NC 27710