Built On Leadership, Vision, And Commitment To Find A Cure

“Think Tank” Meeting
April 5, 2014
San Francisco, California
**Meeting AM Agenda**

**Grand Hyatt San Francisco - Meeting Room – Union Square**  
**Meeting Moderator: Dr. Allison Ashley-Koch**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>Breakfast</td>
</tr>
<tr>
<td>9:00</td>
<td>Welcome - Dorothy Poppe, Executive Director</td>
</tr>
<tr>
<td>9:05</td>
<td>CSF Financial Update - Raymond Cornelius</td>
</tr>
<tr>
<td>9:10</td>
<td>unite@night Walks/CSF Educational Lecture Series - Cathy Poznik</td>
</tr>
<tr>
<td>9:20</td>
<td>2014 Consider Chiari Lecture Series - Lory Watson</td>
</tr>
<tr>
<td>9:30</td>
<td>2014 Bobby Jones Classic - Rob Noble</td>
</tr>
<tr>
<td>9:40</td>
<td>Overview of CSF's Research Strategic Plan - Dr. Rick Batzdorf</td>
</tr>
<tr>
<td>9:45</td>
<td>2013-2015 CSF Hydrodynamic Symposium/IHIWG - Dr. Harold Rekate</td>
</tr>
</tbody>
</table>
| 10:00 | Discussion of 2013 – 2014 Colloquium - Dr. Fraser Henderson  
  * Review of Consensus - Dr. Ulrich Batzdorf, Dr. Edward C. Benzel  
  * Discussion on publication of book |
| 10:15 | Break |
| 10:30 | CSF Grant Recipient Presentations & Other Research Presentations - Dr. Allison Ashley-Koch |
| 10:35 | Mapping Perivascular Spaces in the Spinal Cord Toward Improved Spinal Cord Flow Models - Dr. Malisa Sarntinoranont |
| 11:00 | NIH/NINDS Perspective / CDE Elements - Dr. Joanne Odenkirchen (Skype) |
| 11:25 | Automated MRI-Based Parcellation of the Posterior Cranial Fossa – (Dr. Ahmet Murat Bagci) Presented by Dr. Noam Alperin |
| 11:55 | Development of a Numerical Model of Spinal CSF Dynamics – Dr. Christopher David Bertram - Please see information in PDF/booklet |
Meeting PM Agenda

Afternoon Session Moderator: Dr. Rick Batzdorf

12:00  
Lunch (Room: Sunset A/B)

1:00  
CSF International Patient Registry Project
Steering Committee: Dr. Allison Ashley-Koch, Dr. Ulrich Batzdorf, Dr. David Limbrick, Dr. Mark Luciano, Dr. Brandon Rocque, Dr. Cormac Maher, Dr. Roger Kula, Dorothy Poppe

1:00  
Overview of the Project - Dr. Mark Luciano

1:15  
The Randomized Registry Trial – The Next Disruptive Technology in Clinical Research? (Michael S. Lauer, MD, & Ralph B. D’Agostino, Sr, PhD)- Discussed by Dr. Ulrich Batzdorf

1:30  
Sub-committee Appointments - Dr. Mark Luciano

1:45  
Seven Breakout Groups/Skype

2:45  
Groups back in main meeting room for Committee Chair summaries and final questions

3:15  
Evaluation of gene expression in blood and dura mater from patients with Chiari malformations - (Dr. Christina Markunas)
Presented by Dr. Allison Ashley-Koch

3:45  
Break

4:00  
SEA Board Business – Dr. Allison Ashley Koch / Dr. Ulrich Batzdorf

80  
- Review By-Laws

84  
- Rotate members of SEA Board, per CSF By-Laws
- Rotate Chairman to Senior Advisory Panel, rotate Vice-Chairman to Chairman’s position, and elect new Vice-Chairman (2-year term)
- Elect/rotate Executive Committee members, as needed

85  
- Sign Conflict of Interest Statements

4:30  
Closing of Meeting - Dr. Ulrich Batzdorf

7:00  
Dinner: Jardinière, 300 Grove Street, San Francisco, California
Our Mission, Vision, and Core Values

**Mission:**
To advance knowledge through research and to educate the medical, allied sciences, and lay community about Chiari malformation, syringomyelia and related disorders.

**Vision:**
Within a generation, we will be the premiere world-wide resource for professional and lay people seeking accurate and current information about treatments for and best practices surrounding the management of CM and SM. With our unique resources, both financial and intellectual, we will be the premiere, world-wide resource for accurate and current information surrounding CM, SM and related disorders and the driving force promoting ongoing programs and research focused on earlier diagnosis and better outcomes for people suffering with these disorders.

**Core Values:**
- Honesty and integrity is foremost in everything we do
- Commitment to quality is central to all activities
- Dedication to exceed the expectations of patients and physicians
- Recognition of the critical role played by the Board of Trustees and the Medical Research Board in terms of providing support and guidance to the Board of Directors
- Our volunteers and members are a respected source of knowledge and experience
- Social responsibility
- Fiscal responsibility
Scientific Education & Advisory Board (SEA Board)

Allison Ashley-Koch, PhD  
*Executive Committee Chair*  
Duke University Medical Center  
Durham, North Carolina

Ulrich Batzdorf, MD  
*Executive Committee Vice-Chair*  
David Geffen School of Medicine at UCLA  
Los Angeles, California

Paolo A. Bolognese, MD  
The Chiari Institute  
Great Neck, New York

Richard G. Ellenbogen, MD  
*Executive Committee*  
University of Washington  
Seattle, Washington

Clair Francomano, MD  
*Executive Committee*  
Greater Baltimore Medical Center  
Baltimore, Maryland

David Frim, MD  
University of Chicago Medical Center  
Chicago, Illinois

John D. Heiss, MD  
National Institutes of Health  
Bethesda, Maryland

Fraser Henderson, MD  
*Executive Committee*  
Doctors Community Hospital  
Lanham, Maryland

Bermans Iskandar, MD  
University of Wisconsin Hospitals  
Madison, Wisconsin

Roger W. Kula, MD  
The Chiari Institute  
Great Neck, New York

David Limbrick, MD, PhD  
St. Louis Children’s Hospital  
St. Louis, Missouri

Mark Luciano, MD  
Cleveland Clinic  
Cleveland, Ohio

Dominic J. Marino, DVM, DACVS  
*Executive Committee*  
Long Island Veterinary Specialists  
Plainview, New York

John Oró, MD  
Neurosurgery Center of Colorado  
Aurora, Colorado

Harold Rekate, MD  
*Executive Committee*  
The Chiari Institute  
Great Neck, New York

Marcus Stoodley, MD  
Macquarie University Hospital  
New South Wales, Australia

Shane Tubbs, MD  
University of Alabama at Birmingham  
Birmingham, Alabama
SEA Board Senior Advisory Panel

Edward C. Benzel, MD
Cleveland Clinic
Cleveland, Ohio

Timothy M. George, MD
Dell Children’s Medical Center of Central Texas
Austin, Texas

Barth A. Green, MD
The Miami Project to Cure Paralysis
University of Miami School of Medicine
Miami, Florida

Victor Haughton, MD
University of Wisconsin
Madison, Wisconsin

Arnold Menezes, MD
University of Iowa College of Medicine
Iowa City, Iowa

Misao Nishikawa, MD, Japan
The Chiari Institute
Great Neck, New York
Board of Directors and Staff

**Board of Directors**
- Paul Farrell  
  Chairman
- Joseph Fitzpatrick  
  Vice Chairman
- Raymond Cornelius  
  Treasurer
- Scott Gregerson  
  Director
- Robert Noble  
  Director
- Lory Watson  
  Director at Large

**Staff**
- Dorothy Poppe  
  Executive Director
- Kaitlyn Esposito  
  Program Assistant
- Andrea Grosz  
  Marketing & Development Director
- Cathy Poznik  
  Chapter Coordinator
Board of Trustees

Simon J. Archibald, PhD
Robert Tyre Jones IV, PsyD
Edwin B. Jordan
Adam Korn
Richard E. Kuntz, MD
Lady Trish Malloch-Brown
J. Thomas Megerian, MD, PhD
Michael N. Mikula
Robert E. Rumphrey

Board of Trustees (Emeritus)

Douglas E. Kindlon
Sean Lilienfeld, MD
CSF Old and New Business
## CSF Financial Update

### 2014 Revenue Goals vs 2013 Actual vs 2012 Actual

<table>
<thead>
<tr>
<th>Event</th>
<th>2014 Revenue Goals</th>
<th>2013 Actual</th>
<th>2012 Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night of Light Children’s Gala</td>
<td>$392,500</td>
<td>$390,637</td>
<td>$331,300</td>
</tr>
<tr>
<td>unite@night Walks</td>
<td>$225,000</td>
<td>$149,815</td>
<td>$77,465</td>
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<tr>
<td>Bobby Jones Classic</td>
<td>$163,250</td>
<td>$144,540</td>
<td>$83,050</td>
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<tr>
<td>Charity Ball</td>
<td>$ 63,500*</td>
<td>$46,120</td>
<td>$80,725</td>
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<tr>
<td>Dinner Dance for a Cure</td>
<td>$40,000</td>
<td>$36,865</td>
<td>$27,592</td>
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<tr>
<td>Chicago Event</td>
<td>$125,000</td>
<td>-</td>
<td>$102,465</td>
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<tr>
<td>Direct Mail</td>
<td>$41,500</td>
<td>$31,093</td>
<td>$24,585</td>
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<tr>
<td>Other</td>
<td>$76,900</td>
<td>$38,140</td>
<td>$79,217</td>
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<tr>
<td><strong>Total Revenue</strong></td>
<td><strong>$1,127,650</strong></td>
<td><strong>$837,210</strong></td>
<td><strong>$806,399</strong></td>
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</table>

*$73,000 2014 YTD actual

### 2008 Gross Revenue

$471,217
## CSF 2014 Public Budget

### Revenue:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Direct Public Grants</td>
<td>$30,000.00</td>
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<tr>
<td>Direct Public Support</td>
<td>$5,000.00</td>
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<tr>
<td>Government Grants</td>
<td>$5,000.00</td>
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<tr>
<td>Indirect Public Support</td>
<td>$20,000.00</td>
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<tr>
<td>Program Income</td>
<td>$52,100.00</td>
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<tr>
<td>Special Events Income</td>
<td>$1,009,250.00</td>
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<tr>
<td>Restricted Donations</td>
<td>$5,000.00</td>
</tr>
<tr>
<td>Investments</td>
<td>$1,300.00</td>
</tr>
<tr>
<td><strong>Total Revenue:</strong></td>
<td><strong>$1,127,650.00</strong></td>
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</tbody>
</table>

### Expenses:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Research Grants</td>
<td>$100,000.00</td>
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<tr>
<td>Educational Programs</td>
<td>$100,000.00</td>
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<tr>
<td>Research Programs</td>
<td>$207,788.00</td>
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<tr>
<td>Special Event Expense</td>
<td>$329,603.00</td>
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<tr>
<td>Business Expense</td>
<td>$11,350.00</td>
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<tr>
<td>Contract Services</td>
<td>$34,000.00</td>
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<tr>
<td>Employee Benefit</td>
<td>$18,000.00</td>
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<td>Employee Salary</td>
<td>$240,000.00</td>
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<td>Operations</td>
<td>$63,000.00</td>
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<tr>
<td>Insurance</td>
<td>$11,660.00</td>
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<tr>
<td>Facilities &amp; Equipment</td>
<td>$9,000.00</td>
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<tr>
<td><strong>Total Expense:</strong></td>
<td><strong>$1,124,401.00</strong></td>
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CHIARI & SYRINGOMYELIA FOUNDATION
Statements of Financial Position
As of December 31, 2013 with comparative totals as of December 31, 2012

<table>
<thead>
<tr>
<th>ASSETS</th>
<th>December 31, 2013</th>
<th>December 31, 2012</th>
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<tbody>
<tr>
<td>Current Assets</td>
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<tr>
<td>Citibank Operating Account</td>
<td>$236,328.73</td>
<td>$236,946.48</td>
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<tr>
<td>Capital One Money Market - Restricted</td>
<td>264,936.17</td>
<td>190,108.97</td>
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<tr>
<td>Capital One Money Market - Research</td>
<td>295.23</td>
<td>25,197.74</td>
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<tr>
<td>Capital One Money Market - Reserve</td>
<td>94,197.13</td>
<td>43,918.11</td>
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<td>Total Current Assets</td>
<td>595,757.26</td>
<td>496,171.30</td>
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<tr>
<td>Investments</td>
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<tr>
<td>Goldman Sachs Investment Portfolio</td>
<td>65,054.39</td>
<td>0.00</td>
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<tr>
<td>IntellCell Stock (FMV 12/31/13)</td>
<td>0.00</td>
<td>17,500.00</td>
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<tr>
<td>Total Investments</td>
<td>65,054.39</td>
<td>17,500.00</td>
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<tr>
<td>Fixed Assets</td>
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<tr>
<td>Computer Equipment</td>
<td>8,810.22</td>
<td>8,810.22</td>
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<tr>
<td>Accum Depreciation</td>
<td>(6,203.00)</td>
<td>(4,457.00)</td>
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<tr>
<td>Furniture and Fixtures</td>
<td>579.69</td>
<td>579.69</td>
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<tr>
<td>Accum Depreciation</td>
<td>(413.00)</td>
<td>(302.00)</td>
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<tr>
<td>Total Other Current Assets</td>
<td>2,773.91</td>
<td>4,630.91</td>
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<tr>
<td>Other Current Assets</td>
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<tr>
<td>Undeposited Funds</td>
<td>2,768.17</td>
<td>9,115.14</td>
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<tr>
<td>Total Other Current Assets</td>
<td>2,768.17</td>
<td>9,115.14</td>
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<tr>
<td>TOTAL ASSETS</td>
<td>666,353.73</td>
<td>527,417.35</td>
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<tr>
<td>LIABILITIES AND NET ASSETS</td>
<td></td>
<td></td>
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<tr>
<td>Other Current Liabilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accounts Payable</td>
<td>36.54</td>
<td>6,377.00</td>
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<tr>
<td>Credit Card Payable</td>
<td>4,292.38</td>
<td>106.38</td>
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<tr>
<td>Total Other Current Liabilities</td>
<td>4,328.92</td>
<td>6,483.38</td>
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<tr>
<td>Net Assets</td>
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<tr>
<td>Permanently Restricted Net Assets</td>
<td>251,340.44</td>
<td>251,340.44</td>
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<tr>
<td>Unrestricted Net Assets</td>
<td>408,684.37</td>
<td>269,593.53</td>
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<td>Total Net Assets</td>
<td>660,024.81</td>
<td>520,933.97</td>
</tr>
<tr>
<td>TOTAL LIABILITIES AND NET ASSETS</td>
<td>$664,353.73</td>
<td>$527,417.35</td>
</tr>
</tbody>
</table>
unite@night Walks

unite@night is a one-mile casual evening walk, in various locations around the country and in Canada during the month of June that brings together people who are suffering with the devastating effects of Chiari malformation, syringomyelia and related disorders.

unite@night supports CSF Chapters to provide education to physicians and the lay community and increase awareness, while funding important educational programs and research projects.
Chapters/Educational Lecture Series

Chapters are set up across the United States to offer support for patients and their families. Chapters are also responsible to set up free, educational lectures for patients, their families, and medical professionals, alike.

These lectures are always taped and posted on the CSF website so patients and physicians from around the world have immediate access to this material.

What exactly does a CSF Chapter do?
A Chapter will increase awareness, provide support, institute educational programs, and raise funds in your area.

There have been over 40 educational lectures to date

These lectures are all available online for free access on the CSF website
Consider Chiari Campaign

Some doctors are failing to **Consider Chiari** when patients present textbook symptoms.

Our goal is to educate medical professionals on the symptoms and prevalence of Chiari so that when symptoms present, MRI technology will be utilized and a proper diagnosis can be given.

**Objective:** To spark awareness, putting Chiari on the radar of medical professionals. We want people to **Consider Chiari.**

**Goal:** We want 100% of the participants to leave with a clear picture of the symptoms of Chiari and that proper diagnosis requires an MRI.
The third annual Bobby Jones Classic for CSF will strive to honor, celebrate and exemplify the life and legacy of Bobby Jones. The event's activities will include a private tour of Bobby Jones landmarks, an Alexa Stirling putting competition and a cocktail reception on the evening prior to the tournament. Monday's activities will include a full day of golf at East Lake Golf Club followed by an awards dinner featuring Bob Jones IV, Sid Matthew and Charles Harrison.
Night of Light Gala

Guests gathered on November 9, 2013 at the James Burden Mansion in New York City for this exclusive, white-tie event.

**Gala Honorees:**
Mr. and Mrs. Michael and Denise Mikula, Professor and Mrs. Rodney and Barbara Grahame, Lord and Lady Mark and Trish Malloch-Brown.

**Keynote Speaker:**
Mr. Duncan Niederauer, *Chief Executive Officer and Director, NYSE Euronext.*

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2014 Night of Light Gala
November 22, 2014
Anderson House, Washington DC
Overview of Research Strategic Plan

CSF sponsored this two-day professional research conference on November 11 and 12, 2010 in Chicago, Illinois, which focused on new developments in Chiari research and controversies in diagnosis and care. The event brought together the top physicians and researchers involved with Chiari malformation to share recent developments, discuss and debate controversial topics, and foster collaboration for future work. The proceedings are available through a video web archive.

**CSF Ehlers-Danlos Syndrome Colloquium** (2011)
CSF sponsored the Ehlers-Danlos Syndrome Colloquium which was held on October 1-2, 2011 at the Cosmos Club in Washington DC. Video of the Colloquium can be seen by clicking the link above.

**Project to Analyze the Prevalence of Chiari Malformation Within the ASD Population**
There appears to be an underserved population of children and adults with Chiari malformation and autism – the size of which is yet to be determined. Recent data suggests that a greater than expected overlap of findings in children diagnosed with Chiari Malformation and those diagnosed with Autism Spectrum of Disease (ASD).

**1st CSF Hydrodynamics Symposium** (2011)
On July 8 and 9, 2011, CSF proudly sponsored the first CSF Hydrodynamics Symposium in Zurich, Switzerland, *thanks to a very generous grant from the Monkton Institute and Ms. Candida Lancaster.*

**CSF Think Tank Meeting** (2013) - New Orleans, Louisiana
Members of the CSF SEA Board, Board of Directors, Board of Trustees, and staff attended the annual CSF "Think Tank" meeting, in conjunction with the AANS annual meeting.
Overview of Research Strategic Plan

2nd CSF Hydrodynamics Symposium (2013)
The number of investigators conducting numerical and experimental simulations to better understand the dynamics of cerebrospinal fluid (CSF) has continued to increase since the 1st International CSF Dynamics Symposium held in Zurich, Switzerland. Building on this momentum, we held the 2nd International CSF Dynamics Symposium to continue exchange of ideas toward modeling of CSF.

CSF Research Colloquium (2013)
Colloquium/consensus conference to facilitate a retrospective analysis of patient images to determine whether Spinal Cord Stress Analysis Assessment is helpful in determining which patients may need reductions, fusion, stabilization

CSF Think Tank Meeting (2014) – San Francisco, California
Members of the CSF SEA Board, Board of Directors, Board of Trustees, and staff attending the annual CSF "Think Tank" meeting, in conjunction with the AANS annual meeting.

CSF Research Colloquium (2014) - Boston, Massachusetts

CSF International Patient Registry Meeting (2014) - Boston, Massachusetts

3rd CSF Hydrodynamics Symposium (2015) - Amiens, France

CSF International Patient Registry Project (Ongoing)
Chiari malformation (CM), syringomyelia (SM) and related disorders (RD) affect millions of people worldwide. Starting on the path to improved care can be a daunting task. An important early step is to establish a national patient registry, an invaluable tool for improving the lives of people with CM/SM/RD.
CSF Funded Research Grants

**CSF Funded Grants (2012)**
Upon scientific review by experts from the CSF Scientific Education & Advisory Board, CSF is pleased to fund the following grants which were submitted for review in response to our 2012-2013 Request for Proposals.

- Mapping Perivascular Spaces in the Spinal Cord Toward Improved Spinal Cord Flow Models (PDF) - Dr. Malisa Sarntinoranont

- Automated MRI-Based Parcellation of the Posterior Cranial Fossa - Dr. Ahmet Murat Bagci *(Published paper)*

- Development of a Numerical Model of Spinal CSF Dynamics - Dr. Christopher David Bertram

- Genetic Dissection of Chiari Type I Malformation - Christina Markunas, PhD *(Published paper)*

CSF has also funded a Chiari Fellowship at the Duke Center for Human Genetics.
2ND CSF DYNAMICS SYMPOSIUM
Feinstein Institute For Medical Research, Manhasset, New York
June 24 & 25, 2013

ORGANIZED BY BRYN MARTIN, LYNNE BILSTON, & SHAOKOON CHENG
# SCHEDULE OF EVENTS

**Day 1 – Monday, June 24, 2013**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td><strong>BREAKFAST</strong></td>
</tr>
<tr>
<td>8:30</td>
<td><strong>Opening Remarks</strong></td>
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<tr>
<td></td>
<td><em>Bryn Martin, Lynne Bilston, Shaokoon Cheng</em></td>
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<tr>
<td>8:45</td>
<td><strong>PLENARY TALK</strong></td>
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<tr>
<td></td>
<td><em>Chair: Lynne Bilston</em></td>
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<tr>
<td>9:45</td>
<td>Fine Structure Of CSF And Interstitial Fluid Spaces And Their Drainage Pathways From The Human Central Nervous System</td>
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<tr>
<td></td>
<td><em>Roy Weller</em></td>
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<tr>
<td>10:15</td>
<td><strong>SESSION A: EFFECT OF MICROANATOMY ON CSF</strong></td>
</tr>
<tr>
<td></td>
<td><em>Chair: Lynne Bilston</em></td>
</tr>
<tr>
<td></td>
<td><strong>SESSION A: EFFECT OF MICROANATOMY ON CSF</strong></td>
</tr>
<tr>
<td></td>
<td><em>Chair: Lynne Bilston</em></td>
</tr>
<tr>
<td>9:45</td>
<td>Spinal Cord Nerve Roots And Denticulate Ligaments Alter CSF Dynamics In The Upper Cervical Spine</td>
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<tr>
<td></td>
<td><em>Bryn Martin</em></td>
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<tr>
<td>10:15</td>
<td><strong>Effect Of Spinal Micro-anatomy On CSF Flow Patterns</strong></td>
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<tr>
<td></td>
<td><em>Andreas Linninger</em></td>
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<tr>
<td>10:45</td>
<td><strong>MORNING COFFEE BREAK</strong></td>
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<tr>
<td>11:15</td>
<td><strong>SESSION B: MODELING</strong></td>
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<tr>
<td></td>
<td><em>Chair: Shaokoon Cheng</em></td>
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<td></td>
<td><strong>SESSION B: MODELING</strong></td>
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<tr>
<td></td>
<td><em>Chair: Shaokoon Cheng</em></td>
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<tr>
<td>11:15</td>
<td>CSF Dynamics Society</td>
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<tr>
<td></td>
<td><em>Vartan Kurtcuoglu</em></td>
</tr>
<tr>
<td>11:30</td>
<td>Quantitative Assessment Of The Differences In Spinal CSF Dynamics In Chiari Malformation</td>
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<tr>
<td></td>
<td><em>Frank Loth</em></td>
</tr>
<tr>
<td>12:00</td>
<td>The Spinal Cord And Meninges As A Fluid-filled Elastic Waveguide In Syringomyelia</td>
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<td></td>
<td><em>A. (Tony) Lucey</em></td>
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<tr>
<td>12:30</td>
<td><strong>LUNCH</strong></td>
</tr>
<tr>
<td>13:30</td>
<td><strong>SESSION C: IMAGING</strong></td>
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<tr>
<td></td>
<td><em>Chair: Mark Wagshul</em></td>
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<tr>
<td>13:30</td>
<td>4D MR Flow Imaging: Experiences In Hemodynamics And Potentials In CSF Hydrodynamics</td>
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<td></td>
<td><em>Oliver Wieben</em></td>
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<tr>
<td>14:00</td>
<td>Blood And CSF Flow: What We Can See And What We Would Like To See Soon!</td>
</tr>
<tr>
<td></td>
<td><em>Olivier Balédent</em></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
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</tr>
<tr>
<td>14:30</td>
<td>Novel MRI-based Measurements Of CSF Flow Dynamics In Pediatric Patients With Chiari Malformation</td>
</tr>
<tr>
<td>15:00</td>
<td><strong>AFTERNOON COFFEE BREAK</strong></td>
</tr>
<tr>
<td>15:30</td>
<td>What Role Does CSF Play In Vision Impairment In Astronauts</td>
</tr>
<tr>
<td>16:00</td>
<td>Mathematical Models Of CSF Dynamics: Uses And Challenges</td>
</tr>
<tr>
<td>16:30</td>
<td>Dynamics And Solute Transport In CSF In Non-human Primates As Seen By Positron Emission Tomography</td>
</tr>
<tr>
<td>19:00</td>
<td><strong>SYMPOSIUM DINNER AT 7 PM AT LIMANI</strong></td>
</tr>
</tbody>
</table>

**Day 2 – Tuesday, June 25, 2013**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:30</td>
<td><strong>BREAKFAST</strong></td>
<td></td>
</tr>
<tr>
<td>8:30</td>
<td>Pathogenesis And Pathology Of Hydrocephalus</td>
<td>Marc Del Bigio</td>
</tr>
<tr>
<td>10:00</td>
<td>Biomechanics Of Demyelination Processes: How Shear Wave Propagation Can Reveal Microarchitectural Changes</td>
<td>Ralph Sinkus</td>
</tr>
<tr>
<td>10:30</td>
<td><strong>MORNING COFFEE BREAK</strong></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>How To Use Experimental Data Effectively In Modeling</td>
<td>Lynne Bilston</td>
</tr>
</tbody>
</table>

CONTINUED ON NEXT PAGE
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:30</td>
<td>Near-Wall Ventricular Cerebrospinal Fluid Dynamics</td>
<td>Vartan Kurtcuoglu</td>
</tr>
<tr>
<td>12:00</td>
<td>On The Assumption Of Laminar CSF Flow In The Spinal Canal</td>
<td>Kent-Andre Mardal</td>
</tr>
<tr>
<td>12:30</td>
<td><strong>LUNCH</strong></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td>Potential Cerebrospinal Fluid Flow Pathways In The Development Of Syringomyelia</td>
<td>Shaokoon Cheng</td>
</tr>
<tr>
<td>14:00</td>
<td>Cerebrospinal Fluid And Spinal Cord Morphology Changes In The Hours After Spinal Cord Injury: Results From Novel Porcine Model</td>
<td>Peter Cripton</td>
</tr>
<tr>
<td>14:30</td>
<td>Dynamic Cerebrospinal Fluid Pressure During Experimental Contusion Spinal Cord Injury: Results From Novel Porcine And Synthetic SCI Models</td>
<td>Claire Jones</td>
</tr>
<tr>
<td>15:00</td>
<td><strong>AFTERNOON COFFEE BREAK</strong></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td>A Fractional Pressure-Volume Model Of Cerebrospinal Fluid Dynamics: Marmarou's Model Revisited</td>
<td>Corina S Drapaka</td>
</tr>
<tr>
<td>16:00</td>
<td>A Pilot, Multi-scale Numerical Framework For Brain Mechanics</td>
<td>Diane Dezelicourt</td>
</tr>
<tr>
<td>16:30</td>
<td>Closing Remarks</td>
<td>Bryn Martin &amp; Lynne Bilston</td>
</tr>
<tr>
<td>17:00</td>
<td><strong>DISCRETIONARY PLENARY DISCUSSION AND CLOSING COFFEE</strong></td>
<td></td>
</tr>
</tbody>
</table>
CONSENSUS STATEMENT

1. Ventral brainstem compression, medullary kinking and deformation of the upper spinal cord and/or
brainstem over the odontoid process are potentially deleterious to the brainstem and upper spinal cord.

2. Deformation of the brainstem may manifest clinically as the Cervical Medullary syndrome.

3. The clinical findings of Cervical Medullary Syndrome may include, but are not limited to, the following:
   i) headaches, suboccipital pain and neck pain,
   ii) Bulbar and related symptoms: altered vision, diplopia, nystagmus, decreased hearing, tinnitus,
imbalance, vertigo, dizziness, choking, dysarthria, dysphagia dysautonomia, postural orthostatic
tachycardia, pre-syncopal or syncopal episodes disordered sleep architecture, sleep apnea,
   iii) Symptoms of myelopathy: weakness, clumsiness, spasticity, altered sensation, paresthesias,
dysesthesia, change in gait, constipation, urinary urgency and frequency

4. In assessing the potential for cranio-cervical instability, it is reasonable to measure the angle between
the clivus and the spine. This angle has been termed the clivus canal angle, the clivus vertebral angle,
the clivus spinal angle, the clivus cervical angle and the clivus-axial angle.

   In keeping with the greater part of the literature, we recommend the uniform adoption of the term
clivo-axial angle. This angle may be abbreviated CXA.

5. The clivo-axial angle is the angle between the clivus line and the posterior axial line. The clivus line is
drawn along the lower third of the clivus - from the spheno-occipital synchondrosis to the basion, or in
the case of basilar invagination, the superior most aspect of the odontoid.

   When assessing the CXA with sagittal CT scan or X-ray, the posterior axial line may be drawn along the
posterior edge of the odontoid.

   When assessing the CXA with MRI, the posterior axial line should be drawn from the posterior edge of
the tectorial membrane to the inferior posterior edge of the posterior ligament of the C2 vertebra.

   The CT and MRI measurements may differ in the same patient: the CXA determined by CT reflects the
more traditional means of measurement; the CXA determined by MRI will necessarily include thickening
of the ligament due to pannus.

6. The literature suggests that a clivo-axial angle of 135 degrees or less is potentially pathological. That is
a CXA of 135 degree, may in some circumstances, result in harmful deformative stress upon the
brainstem and upper spinal cord and, therefore, warrants consideration for further evaluation and
possible treatment.
7. The CXA can be measured on sagittal CT or MRI, with the patient assuming moderate flexion of the cranio-cervical junction. If a flexion view is not available, a neutral position will suffice in most circumstances. An upright dynamic MRI may be desirable in some circumstances – but, such is often not available.

8. In assessing the potential for cranio-cervical instability, it is reasonable and appropriate to measure the BpC2 line, also known as the Grabb-Oakes measurement or line, or the Grabb Mapstone Oakes Measurement, as one method to approximate the potential presence and magnitude of ventral brainstem compression. We use the term Grabb-Oakes measurement herein. The Grabb-Oakes measurement is the distance in millimeters from the dura to the line drawn from the basion to the posterior inferior edge of the C2 vertebra. A Grabb Oakes measurement of 9mm represents the diagnostic threshold for ventral brainstem deformity. Some clinicians may choose 8 mm as the diagnostic threshold at which there may is potential ventral brainstem deformity.

9. The Harris measurement, also known as the Basion Axial Interval (BAI = distance from tip of basion to posterior axial line), when drawn horizontally, should be less than 12 mm. The basion to dens interval (BDI = distance from basion to tip of odontoid) drawn vertically, should be less than 12 mm. The posterior axial line should be drawn along the posterior ligamentous surface of the C2 vertebra. In keeping with the literature, a Harris measurement exceeding 12 mm is considered potentially pathological, and reflects cranio-cervical instability.

10. In the presence of known ligamentous instability, such as a hereditary hypermobility connective tissue disorder, the BAI (the Harris measurement) may be measured with the cervical spine in the flexion and extension positions. This will assess and quantify translation of the basion with respect to the dens (odontoid process). In keeping with the literature, any translation noted on dynamic imaging that exceeds 2mm (the delta BAI > 2 mm), will be considered abnormal and potentially pathological.

11. Cranio-cervical hypermobility is common, and defined by the presence of hyper-extensibility of the connective tissue, and in particular, hyper extensibility of the joints. While hypermobile joints occur frequently in healthy children, such can also be severely disabling in others. Ehlers Danlos syndrome, cleidocranial dysostosis, Down syndrome, Marfan syndrome, Morquio syndrome and several other less well known connective tissue disorders are associated with ligamentous laxity. A pathological Lax Ligament Syndrome may result in cranio-vertebral instability, kyphosis of the clivo-axial angle and ventral brainstem compression.

The growing body of knowledge regarding the prevalence of hypermobility connective tissue disorders should lead to more widespread recognition of the impact of ligamentous laxity on the health of sufferers of hypermobility syndromes.
CSF Grant Recipient Presentations
Mapping Perivascular Spaces in the Spinal Cord towards Improved Spinal Cord Flow Models

Specific Aims: Perivascular spaces have been implicated as a significant fluid source for syringomyelia [1]. The spatial complexity of these spaces can be profound; however, few studies have attempted to quantify the spatial variation of these spaces and the influence of their shape on underlying fluid transport. We hypothesize that the major changes in perivascular dimensions and complexity will occur over several length scales (mm to micron) and have a major impact on fluid resistance, pressure, and flow through these channels. Improved spatial representation of the perivascular spaces is key towards determining the fluid dynamics of these spaces and their relative contribution to fluid flow within the spinal cord. In this study, we propose to systematically map perivascular spaces within the rat spinal cord and determine the influence of a transient increase of cerebrospinal fluid (CSF) pressure on perivascular fluid dynamics.

Specific aim 1: In vivo tracer distributions in the spinal cord under varying CSF pressure. Intraventricular infusions of macromolecular protein tracers (such as Evans Blue albumin) will be designed to minimally disrupt fluid flow within the spinal cord and limit tracer penetration to major fluid pathways including perivascular spaces. CSF pressure will be increased by infusing controlled volumes of artificial CSF in excess of normal capacity, and changes in perivascular tracer distributions under conditions of normal and elevated CSF pressure (two control groups) will be determined.

Specific aim 2: Map 3D perivascular spaces. Following in vivo studies, fixed tissue slices will be imaged to quantify tracer spread along perivascular spaces using optical microscopy. We will reconstruct the interconnected network of perivascular spaces within a small representative tissue section ~1 mm x 1 mm x 1 mm. Slice-by-slice geometric reconstruction of these regions will provide 3D geometries that correspond to the interconnected perivascular spaces over 10 to 100 micron length scales. These geometries will be imported into preliminary computational fluid dynamic models to determine the relative effects of diffusion, convection and active pumping transport processes.

While conditions for syrinx formation will not be specifically recreated in this study, perivascular staining will be due to normal function as well as under a high pressure condition. Completion of these specific aims are a first step towards quantifying underlying 3D fluid flow pathways which can provide a mechanism for syrinx formation under certain pathological conditions. Also, these data will be used as feasibility data for larger scale studies involving animal models of syringomyelia. Development of computational models that use these perivascular geometries will also be useful for simulating conditions that promote enhanced perivascular transport.

Rationale: Perivascular spaces (also known as Virchow-Robin spaces) are open fluid spaces that exist around major cerebral blood vessels interior to the arachnoid membrane that surrounds the CNS, see Fig. 1. Previous spinal cord studies have shown have measured tracer distributions within perivascular spaces in animal models of syringomyelia and have implicated perivascular transport as one pathway for fluid into syrinxes [2-4]. A number of analytical and computational models have also investigated the underlying fluid dynamic pumping mechanisms [5, 6]. These models usually assume simple cylindrical geometries or treat these spaces as a black box. However, flow resistance through channels is extremely sensitive to channel dimensions (gap thickness) and it is expected that as arteries branch and their diameter progressively decreases, corresponding perivascular spaces will also change greatly in dimensions, see Fig. 2. 3D maps of changes in perivascular space fill in a critical gap in knowledge related to 3D flow resistance. Resistance is necessary for predicting pressure gradients required to move fluid from outer CSF to internally connected parenchymal space and syrinx.

Figure 1. Schematic of the perivascular space [4].
Our group has experience in CNS tracer infusion studies and we are uniquely positioned to create experimentally-driven models of spinal cord fluid dynamics related to syringomyelia and syrinx formation. We have extensive experience quantifying CNS tissue distributions in 3D as well as in developing computational transport models of both the spinal cord and brain. These models treat tissue as a porous media in which extracellular (interstitial) fluid communicates with CSF fluid. We have tracked tracer distributions following direct infusions into the brain hippocampus as well as intrathecally following peripheral nerve injection (see attached publications).

**Research Plan:**

**In vivo tracer infusions:** Adult female Sprague-Dawley rats (~250 g) will undergo intraventricular infusions of the macromolecular tracer, albumin tagged with Evans Blue. This tracer was selected because it is not taken up by cells, is sufficiently large (~64 kDa) to restrict diffusion into the bulk of brain tissue, but small enough to move into perivascular spaces.

Rat surgery will be conducted in accordance with the NIH guidelines on the use of animals in research and the regulations of the Animal Care and Use Committee of the University of Florida. Two control groups (n=10 for each group) corresponding to high and normal intracranial pressures will be tested. Rats will be anesthetized and the macromolecular tracer will be infused into the rat brain ventricle. Infusion pressures will be continuously monitored using an in-line system previously used by our lab. For the control group with elevated intracranial pressure, a volume of CSF fluid will be infused prior to tracer infusions to increase ICP to the desired level. The ventricle infusion site was chosen so as to minimize disruption to fluid flows in the spinal cord region. With sufficient time, tracers should spread throughout all interconnected CSF spaces including around the spinal cord and into the perivascular spaces. Tracer concentrations, volumes and post infusion times that optimize tracer enhancement in the spinal cord will be determined in initial testing. If insufficient quantities of tracers reach the spinal cord, we will consider changing sites of infusion (e.g., cisterna magna or third ventricle) or tracer (horseradish peroxidase). A sufficient time after infusions for tracer to distribute within the perivascular space (10 min to 1 hr), rats will be euthanized and undergo perfusion fixation.

**3D mapping:** To quantify tracer penetration into perivascular spaces, excised spinal tissues within cervical to thoracic regions of the spinal cord will be cut into 10-50 micron slices, mounted onto slides, and imaged over a 1 mm

\^3

section. Commercial (Amira) as well as custom image analysis software will be used to separate out Evans blue dye regions in each slice. Slice-by-slice geometric reconstruction of these regions will provide 3D geometries that correspond to the interconnected fluid spaces of the perivascular network over micron to mm length scales. We will not reconstruct all perivascular space, but will focus on a region near penetrating branches of the anterior and posterior spinal arteries. We will also observe overall tracer distribution patterns in the spinal cord and along peripheral nerves entering the spinal cord. Interconnected fluid spaces will be incorporated in preliminary fluid dynamic models which account separately for diffusion, convection, and active transport to determine what conditions result in tracer penetration seen experimentally.

**Future work and funding plan:** These data will provide the basis for futures studies in which perivascular property changes with syringomyelia will be compared. Research results will be incorporated in a NIH proposal which will focus on developing comprehensive spinal cord transport models for understanding mechanisms of syringomyelia. Our ultimate goal is to quantify the relative effects of perivascular transport and extracellular flows (e.g. edema) for improved understanding of the mechanisms of syrinx formation.

![Fig 2. (Top) Nanoparticle tracers (red) in perivascular spaces around a penetrating arteriole and branching capillary (blood vessels shown in green) [7]. (Bottom) Tracer along the wall of a spinal cord vessel [4].](image)
References

Goals and Timeline
The goal is to establish neurological disorder specific Common Data Element (CDE) Working Groups (WGs) in order to recommend disorder/disease-specific elements commonly used in clinical research studies. We anticipate that the disorder/disease specific CDEs will be drafted by WGs and either ready to share with the other WGs or ready to share with the broader research community in one year after initial meetings. The Working Groups will have an opportunity to review feedback from the research community prior to the posting of version 1.0 of the specific disorder/disease CDEs. We also consider in-person meeting(s) at national or international to facilitate this process.

End Products
Each CDE Working Groups should ultimately provide the NINDS CDE Team with the following products:

1. Dictionary of CDEs that includes these specifications for each CDE (see attachment):
   a. Name
   b. Definition
   c. Code list/Permissible values
   d. Definitions for Code list/Permissible values (PVs)
      i. Additional specifications for the PV (e.g., min and max values, datatype, units)
   e. Comments/Special instructions
   f. Reference(s)
   g. Classification (1=Core, 2=Supplemental, 3=Exploratory)*

2. Case report forms (CRFs) for the CDEs

3. List of recommended standardized instruments**, including these details for each instrument:
   a. Name of Instrument
   b. Short description
   c. Scoring
   d. Comments/ Special Instructions
   e. Copyright Information
   f. Reference(s)
   g. Classification (1=Core, 2=Supplemental, 3=Exploratory)*

4. Manual of Procedures (where applicable)

5. Summary of Recommendations

* Classifications

** Core CDE: Used by the majority of the disorder/disease specific studies, strongly encouraged for use in a study

** Supplemental CDE: Used by a significant amount of disorder/disease specific studies, not as crucial to have in a study compared to Core CDEs as their relevance depends upon the study design (i.e., clinical trial, cohort study, etc.) or type of research involved

Within Supplement CDEs are annotated in recommendations for the following:

Highly Recommended: For outcome measures these should have excellent psychometric properties and clinical utility
**Recommended:** For outcome measure, it has good psychometric properties and good clinical utility.

**Exploratory CDE:** Will likely be used by the disorder/disease specific studies but the data elements require further validation before they are ready for prime-time use. Reasonable to use, but limited study in target group; the outcome measure has good or excellent psychometric properties and clinical utility in a related population, but insufficient study in target population to support higher recommendation.

---

**Copyrighted Instruments**

The working groups may recommend copyrighted instruments; however, at the current time the instruments may not be posted on the public [Web site](#). We are currently reaching out to the publishers to find out if we may post the instruments with specific guidelines; however, this process will take some time.

**Development Process**

The NINDS CDE Team will do their best to provide suggestions and assistance throughout the development process and will assist in the development of the end products, in conjunction with the critically important scientific expertise of the WGs.

It is recommended that you address the use of different instruments for different visit types (e.g., which instruments would be used for screening versus longitudinal follow-up versus a single assessment).

In addition, standardized instruments should be classified for their use in different datasets: the basic minimum dataset (e.g., conducted at each visit, similar to the Core CDEs), a comprehensive dataset (similar to the supplemental CDEs), or a dataset for a defined area of interest (e.g., electrodiagnostics).

Each WG should consider any efforts that have already been undertaken to standardize how research and/or clinical data are collected about the disorder/disease as the CDE Project does not want to "reinvent the wheel" but rather strives to reuse relevant work that has already been done in this area.
### Guidelines
Developing Common Data Elements for Neurological Disorders and Stroke

#### Template: CDE Data Dictionary for Elements

<table>
<thead>
<tr>
<th>CDE Name</th>
<th>Definition / Description</th>
<th>Code List / Permissible Values</th>
<th>Definitions for Codes / Permissible Values</th>
<th>Permissible Values Additional Fields</th>
<th>Additional Comments / Special Instructions</th>
<th>References</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the data element being recommended as a CDE</td>
<td>Definition and/or description of the CDE</td>
<td>Describes the standard way the CDE should be coded</td>
<td>Further defines the code list / permissible values</td>
<td>(e.g., Datatype; Min. and Max. Values; Units; and Format)</td>
<td>Other important information about the CDE that will help ensure it is collected consistently</td>
<td>References that contain additional information about the CDE and/or were used to define the CDE</td>
<td>1 = Core; 2 = Supplemental; 3 = Exploratory</td>
</tr>
</tbody>
</table>

#### Template: CDE Data Dictionary for Instruments

<table>
<thead>
<tr>
<th>Instrument / Scale Name</th>
<th>Description</th>
<th>Scoring Information</th>
<th>Comments / Special Instructions</th>
<th>Copyright Information</th>
<th>References</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name and acronym of the instrument/measure that is recommended for inclusion in the CDE</td>
<td>Brief description of the instrument / measure</td>
<td>Total range of scores and range of subscales if appropriate</td>
<td></td>
<td>Explains whether the instrument / measure has copyright protection and if so, provides information on how to obtain it from the publisher</td>
<td>References that contain additional information about the CDE and/or were used to define the CDE</td>
<td>1 = Core; 2 = Supplemental; 3 = Exploratory</td>
</tr>
</tbody>
</table>
## Example: Data Dictionary for Elements (Stroke CDEs)

<table>
<thead>
<tr>
<th>CDE (Listed here as Suggested Question Text) for data element being recommended as a CDE</th>
<th>Definition / Description</th>
<th>Code List / Permissible Values</th>
<th>Definitions for Codes / Permissible Values</th>
<th>Additional Comments / Special Instructions</th>
<th>References</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA procedure initiated</td>
<td>Indicate if an Intra-arterial (IA) procedure was initiated at this hospital.</td>
<td>Yes; No</td>
<td>Yes; No</td>
<td>IA procedures include all uses of IA thrombolytic therapy, as well as mechanical devices such as &quot;Clot retrieval devices&quot;. Mechanical devices may be used alone or in conjunction with IA thrombolytic therapy.</td>
<td>Get With The Guidelines (GWTG) Stroke Patient Management Tool Coding Instructions (Updated on 11/4/2009); Paul Coverdell National Acute Stroke Registry</td>
<td>1</td>
</tr>
<tr>
<td>Type of IA procedure</td>
<td>If yes, specify IA procedure performed at this hospital.</td>
<td>Pharmacological; Mechanical; Both</td>
<td>Pharmacological; Mechanical; Both</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date and Time of Groin Puncture for IA procedure</td>
<td>Indicate date and time of groin puncture</td>
<td>MM/DD/YYYY HH:MM; Unknown</td>
<td>The preferred format for recording date and time is MM/DD/YYYY HH:MM (24-hour clock). 99/99/9999 can be used to indicate an unknown date. Similarly, 99:99 can be used to indicate an unknown time.</td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>
## Example: Data Dictionary for Instruments (Stroke CDEs)

<table>
<thead>
<tr>
<th>Instrument / Scale Name</th>
<th>Description</th>
<th>Scoring Information</th>
<th>Comments / Special Instructions</th>
<th>Copyright Information</th>
<th>References</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Independence Measure (FIM)</td>
<td>The FIM measures degree of independence in activities of self-care, sphincter control, transfers, locomotion, communication, and cognition.</td>
<td>Scores range from 1 (total or &gt;75% assistance) to 7 (complete independence). The total of the 18 items is the patient's total score, which ranges from 18-126. Scores may be used raw or converted to interval scores.</td>
<td>The alpha FIM is a subset that has been used in the acute patient setting to assess which patients are appropriate for discharge to a rehabilitation setting. The alpha FIM may be worth exploring in Phase III trials that include assessments of appropriateness of different post-discharge destinations.</td>
<td>The FIM is proprietary. For further information about obtaining the scale, syllabus, and training materials please contact: Uniform Data System for Medical Rehabilitation 270 Northpointe Parkway, Suite 300 Amherst, New York 14228 (716) 817-7800 FAX (716) 568-0037 email: <a href="mailto:info@udsmr.org">info@udsmr.org</a> Web site: <a href="http://www.udsr.org/Web">http://www.udsr.org/Web</a> Modules/FIM/Fim_About.aspx</td>
<td>Granger CV. The emerging science of functional assessment: our tool for outcomes analysis. Arch Phys Med Rehabil 1998;79(3):235-240. Wright, J. (2000). The FIM(TM). The Center for Outcome Measurement in Brain Injury. <a href="http://www.tbims.org/combi/FIM">http://www.tbims.org/combi/FIM</a> (accessed March 10, 2010).</td>
<td>2</td>
</tr>
</tbody>
</table>

Copyright Information Explains whether the instrument / measure has copyright protection and if so, provides information on how to obtain it from the publisher.
NINDS expects the clinical research it funds to meet the highest standards of scientific rigor yet appreciates the burden that extensive data collection puts on investigators and study participants. Further, the Institute leadership recognizes that investigators independently identify data elements and forms for each study, many of which could be common across studies.

As part of its effort to facilitate research of the highest quality, yet streamline clinical trial data collection in neurological studies, NINDS continues to advance its Common Data Element (CDE) Project. The CDE Project seeks to standardize the way data are collected across the neuroscience research community. Central to the project is the identification of common definitions and terminology.

By identifying CDEs in a standardized format, and by developing common documentation and case report forms, the NINDS hopes to facilitate the development of data collection tools, reduce study start-up time, promote systematic data collection, improve data quality, and facilitate data sharing across clinical research studies.

What do you need to know to use the CDEs?

2. Contact the NINDS Project Officer, Joanne Odenkirchen, (odenkirj@ninds.nih.gov) to set up a time to discuss using the CDEs and to arrange for technical assistance.
3. Share your final or near final protocol with NINDS so the Institute and its contractor can tailor a CDE training session to your study.
4. Send the NINDS Project Officer and the contractor (NINDSCDE@emmes.com) any initial questions or comments you may have about the CDEs or the Web site prior to your scheduled training session.

Review of CDE Terminology:

1. "General" Common Data Elements (CDEs)
   Relevant across neuroscience clinical research

2. Disease-specific Core CDEs
   should be used in all studies for this disease

3. Supplemental Disease-specific CDEs
   extended set that are "common", but supplemental, i.e. not required - choose from a "menu"

4. Exploratory Disease-specific Data Elements
   not yet validated, or under development

Repository Forms or links to other repositories
Which CDEs should you use in your study?

1. Strongly encouraged to use the General Core CDEs.
2. Pick Additional Supplemental and Exploratory CDEs that are applicable to your study.
3. Add data elements not found in the CDEs that are required for your study.

_NINDS CDE Project promotes parsimonious data collection._

Tips for Accessing the CDEs


The NINDS CDE Web site has four main locations to access the CDEs:


Submitting Feedback

We want to hear your feedback and are open to receiving it in a variety of formats:

- **Email** – [NINDSCDE@emmes.com](mailto:NINDSCDE@emmes.com) for NINDS CDE Team; [odenkirj@ninds.nih.gov](mailto:odenkirj@ninds.nih.gov) for NINDS CDE Project Officer.
- **Phone/Webinar** – arrange follow-up teleconference/webinar to review your comments.

If you have general questions about the CDE Project or more specific questions about the development of CDEs for a certain disease area please contact the NINDS CDE Project Officer, Joanne Odenkirchen at [odenkirj@ninds.nih.gov](mailto:odenkirj@ninds.nih.gov)
Automated Posterior Cranial Fossa Volumetry by MRI: Applications to Chiari Malformation Type I

A.M. Bagci, S.H. Lee, N. Nagornaya, B.A. Green, and N. Alperin

ABSTRACT

BACKGROUND AND PURPOSE: Quantification of PCF volume and the degree of PCF crowdedness were found beneficial for differential diagnosis of tonsillar herniation and prediction of surgical outcome in CMI. However, lack of automated methods limits the clinical use of PCF volumetry. An atlas-based method for automated PCF segmentation tailored for CMI is presented. The method performance is assessed in terms of accuracy and spatial overlap with manual segmentation. The degree of association between PCF volumes and the lengths of previously proposed linear landmarks is reported.

MATERIALS AND METHODS: T1-weighted volumetric MR imaging data with 1-mm isotropic resolution obtained with the use of a 3T scanner from 14 patients with CMI and 3 healthy subjects were used for the study. Manually delineated PCF from 9 patients was used to establish a CMI-specific reference for an atlas-based automated PCF parcellation approach. Agreement between manual and automated segmentation of 5 different CMI datasets was verified by means of the t test. Measurement reproducibility was established through the use of 2 repeated scans from 3 healthy subjects. Degree of linear association between PCF volume and 6 linear landmarks was determined by means of Pearson correlation.

RESULTS: PCF volumes measured by use of the automated method and with manual delineation were similar, 196.2 ± 8.7 mL versus 196.9 ± 11.0 mL, respectively. The mean relative difference of −0.3 ± 1.9% was not statistically significant. Low measurement variability, with a mean absolute percentage value of 0.6 ± 0.2%, was achieved. None of the PCF linear landmarks were significantly associated with PCF volume.

CONCLUSIONS: PCF and tissue content volumes can be reliably measured in patients with CMI by use of an atlas-based automated segmentation method.

ABBREVIATIONS: CMI = Chiari malformation type I; PCF = posterior cranial fossa

The current radiologic definition of CMI is based on the degree of tonsillar herniation below the foramen magnum. However, imaging data with x-ray, CT, and MR gathered over the last several decades documented that CMI is also associated with a smaller than normal PCF. In most studies, length (1D) and area (2D) measurements of certain PCF landmarks manually delineated on x-ray film or on a midsagittal MR imaging were used for estimates of the PCF size. Volumetric (3D) assessment of the PCF, either with CT or MR imaging, further confirmed reduced PCF volume in patients with CMI compared with healthy controls. Through the use of manual delineation of the PCF and the brain tissue boundaries, Milhorat et al reported a smaller PCF volume as well as CSF volume, whereas the hindbrain volume was normal, leading to the notion of an overcrowded PCF. In a more recent study, they reported a small PCF volume only in “classic” CMI but not in “CMI mimicking” etiologies, thereby emphasizing the importance of the PCF volume for differential diagnosis of tonsillar herniation.

Lirng et al used manual delineation of the PCF in MR imaging followed by image intensity-based segmentation of brain tissue and CSF to assess the effect of age and sex on the PCF volume and crowdedness in healthy subjects. They found that overall, men had a larger PCF and hindbrain volume, whereas women demonstrated a higher degree of crowdedness, which may explain the higher frequency of CMI in women.

Two other studies further suggest that the size of the PCF is also a strong predictor for surgical treatment outcome in CMI. Badie et al reported that a smaller ratio of the PCF volume relative to the supra-
tentorial volume is associated with a better surgical outcome. A more recent study by Noudel et al\textsuperscript{10} used a semi-automated method to demonstrate that the response to the PCF decompression surgery is correlated with preoperation volume of PCF and the overall increase in the PCF volume after operation but not with the degree of tonsillar herniation or other tested morphologic measures. These limited data suggest that measurements of the PCF volume are likely to enhance both the diagnostic and prognostic reliability in CMI.

Despite the potential diagnostic and prognostic values of the PCF morphology, volumetric assessments of the PCF size are not commonly used in clinical practice because manual delineation of the PCF on multiple images is time-consuming. Manual length measurements of different landmarks of the PCF are less time-consuming and are more commonly used as surrogate measures of the PCF volume.\textsuperscript{11} The most common 1D measurements are the lengths of the supraocciput and the clivus bones and the McRae and Twining lines at the midsagittal plane.\textsuperscript{4,6} However, these measurements are highly subjective and are strongly influenced by the MR imaging technique. Furthermore, it is not clear how well these linear (1D) measures correlate with the overall volume of the PCF. With the increasing evidence for the diagnostic potential of PCF measurement, there is a need for a robust automated method for reliable segmentation of the PCF. Moreover, because MR imaging is the primary technique used for diagnosis of CMI, it is important that such a method is available for MR imaging data.

A new approach for automated PCF parcellation is presented. The proposed parcellation uses atlas-guided segmentation, which has been successful in other cerebral regions. In addition to a measurement of the entire PCF volume, the method also provides measurements of the hindbrain tissue and CSF volumes. The robustness of the method is assessed by comparison with manual delineation in CMI data. Additionally, the degree of association between PCF volumes and linear landmarks is assessed.

**MATERIALS AND METHODS**

**Subjects**

PCF volumes were measured by use of MR imaging data from 3 healthy subjects who were scanned twice on 2 separate days (1 woman; age range, 29–36 years; mean age, 34 ± 3 years) and 5 symptomatic patients with CMI (3 women; age range, 23–48 years; mean age, 37 ± 10 years). MR imaging data from an additional 9 symptomatic patients with CMI (7 women; age range, 20–68 years; mean age, 37 ± 15 years) were used to create the CMI-specific atlas. All patients had cerebellar tonsillar herniation of at least 5 mm below the foramen magnum and presented with suboccipital headaches and numbness in the upper and/or lower extremities. Six patients had Valsalva-induced headaches. All subjects provided written informed consent, and the study was approved by the institutional review board.

**MR Image Acquisition**

The MR images used in the study were acquired with a 3T scanner (Magnetom Trio; Siemens, Erlangen, Germany). The structural analysis was performed on 3D T1-weighted image (magnetization prepared rapid acquisition of gradient echo) with the following acquisition parameters: TR/TE/TI of 1900/2.89/900 ms, flip angle of 9°, FOV of 25.6 × 25.6 cm, and matrix size of 256 × 256, resulting in 1-mm isotropic resolution. Images were acquired in the sagittal orientation.

**Linear Measurements of the PCF**

Lengths of 6 midsagittal PCF structures were measured on the midsagittal T1-weighted image by a trained expert with 5 years of experience (S.H.L.). These structures were characterized as follows: 1) McRae line measured from the basion and the opisthion, 2) clivus length measured from the basion to the inferior boundary of the dorsum sellae, 3) Twining line measured from the dorsum sellae to the internal occipital protuberance, 4) height of the cerebellum, 5) supraocciput measured from internal occipital protuberance to opisthion, and 6) tonsillar herniation measured from the McRae line to tip of the cerebellar tonsil. An example of these markers overlaid on a midsagittal T1 MR image is shown in Fig 1.

**Manual Segmentation of PCF Volume**

The PCF was manually outlined on every sagittal section by use of the 3D Slicer software (http://www.slicer.org) by a single trained expert (A.M.B.) to avoid interobserver variability. Manual delineations were further reviewed and edited when needed by a neuroradiologist (N.N.). The PCF was anatomically bounded by tentorium cerebelli, occipital bone, clivus, and foramen magnum. The volume of the PCF was calculated by summation of the volume of each voxel within the manually created mask on each sagittal section.

**CMI-Specific PCF Atlas**

A PCF reference atlas, specific for CMI, was created from T1-weighted images of 9 patients (7 women; age range, 20–68 years; mean age, 37 ± 15 years). PCF labels were manually delineated on each subject image by an expert (A.M.B.) and were reviewed and confirmed for reliability by a neuroradiologist (N.N.). The T1-weighted images were then affine-registered to Montreal Neurological Institute 152 space\textsuperscript{12} and averaged to create the atlas template. The delineated PCF region from each of the 9 subjects was

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**FIG 1.** The 6 linear landmarks of the PCF superimposed on a midsagittal T1-weighted MR imaging from a patient with CMI: herniation (HR), McRae line (MC), clivus (CL), Twining line (TW), cerebellum (CR), and supraocciput (SO).
then projected to the atlas space to generate an averaged PCF mask superimposed on the atlas space. The average reference atlas and the superimposed PCF regions are shown in Fig 2.

Automated Segmentation of PCF Volume
Automated segmentation of the PCF volume is achieved by use of the previously described CMI reference atlas. First, a global linear transformation is applied to register the atlas template to the subject dataset by use of the FLIRT tool from FSL software package (http://www.fmrib.ox.ac.uk/fsl). Only the brain region is used for the global registration to avoid adverse effects of cranial and extracranial structures on registration. After the global linear transformation, a more precise local alignment with a nonlinear registration is achieved by use of the FMRIB Nonlinear Image Registration Tool (FNIRT) from FSL, which is based on minimizing a sum-of-squares cost function by use of a Levenberg-Marquardt modification of the Gauss-Newton method. Finally, the PCF mask is mapped to the subject MR imaging through the inverse of the registration to automatically segment the PCF volume.

Automated Segmentation of Brain Tissue
The proposed PCF parcellation method quantifies the volumes of tissue content of the PCF, which include the brain stem, cerebellum, medulla, and pons. Each pixel inside the PCF mask is labeled as gray matter, white matter, or CSF, by use of an algorithm that is based on the hidden Markov random field model and expectation maximization. The cerebellar tonsils extending beyond the foramen magnum are excluded as the result of PCF masking. A flow chart of the FSL implementation of the process for PCF segmentation and measurement of the PCF tissue volume is shown in Fig 3.

The intensity-based segmentation of the PCF tissues was compared with FreeSurfer-based (http://surfer.nmr.mgh.harvard.edu) segmentation of the hindbrain. The details of the FreeSurfer segmentation method are provided by Fischl et al. FreeSurfer segmentation is an atlas-based segmentation method, which used both the intensity distribution and the spatial relationships of previously defined brain regions. Labeled brain regions in the brain stem and gray and white matter in left and right cerebellum were combined and used as the hindbrain volume in this study.

Assessment of Automated Segmentation Accuracy and Reliability
The accuracy of the automated segmentation was assessed by comparing the automatically segmented PCF with manual seg-
mentation in 5 patients with CMI. Percentage volume difference between the manual and the automated segmentations were calculated to test for systematic differences. Additionally, the Dice similarity coefficient was used to evaluate the degree of spatial overlap between the automatically and manually segmented PCF. The Dice similarity coefficient is defined as

$$DSC = \frac{2 \times V(A \cap M)}{V(A) + V(M)}$$

where $A$ and $M$ denote automated and manual segmentations, and $V$ denotes volume of the region. The value of Dice similarity coefficient ranges from 0–1, representing no overlap to complete spatial overlap, respectively. A Dice similarity coefficient value $>0.7$ is considered as a good agreement between 2 compared measurements.18

The reliability of the automated segmentation across scan sessions was tested by means of MR imaging data from the 3 healthy subjects who were scanned twice on 2 separate days. The mean absolute percentage difference of the 2 measurements was calculated by dividing the absolute difference of the 2 measurements by their mean. A paired, 2-tailed $t$ test was applied to determine the significance of differences between volume measurements obtained by use of 2 methods.

The posterior fossa brain tissue volume was measured in 5 patients with CMI by use of the proposed automated method and compared with measurements obtained by means of FreeSurfer. The mean percentage difference between 2 measurements and degree of spatial overlap was calculated. In addition, the derived crowdedness index, defined as the ratio of the tissue volume and the PCF compartment volume, were compared as well. Finally, linear association between PCF volumes and the length of the 6 different linear PCF markers were assessed by calculating the Pearson correlation coefficient and significance level by use of the data from the 14 patients with CMI. All statistical calculations were performed by use of MedCalc statistical software (MedCalc, Mariakerke, Belgium).

RESULTS

**PCF Volumes**

Examples of midsagittal and axial images with the identified PCF boundary and the 3D-rendered PCF volume from a representative patient with CMI are shown in Fig 4. The PCF volumes of each of the 5 patients with CMI measured with the 2 methods and the Dice coefficient representing the degree of overlap are shown in Fig 5. The mean and the SD of the manual and the automated segmentations of the PCF volumes in these 5 patients with CMI were similar (197 ± 11 mL and 196 ± 9 mL, respectively). The difference was not significant ($P = .7$). The mean percentage volume difference was $-0.3 \pm 1.9\%$. The mean degree of overlap (Dice coefficient) between the automatically and manually obtained PCF masks was 0.96 ± 0.001.

The PCF volumes measured from the 3 healthy subjects who were scanned twice on 2 separate days and the relative percentage difference are listed in Table 1. The mean absolute percentage difference was $0.6 \pm 0.2\%$, with a range of $0.4\%$ to $0.8\%$; the difference between volume measurements was not statistically significant ($P = .995$).

**PCF Crowdedness Indexes**

The posterior fossa brain tissue volume, crowdedness indexes of the 5 patients with CMI measured with the proposed method and with FreeSurfer, and the Dice coefficient are listed in Table 2. The mean and SD of the volumes measured with the proposed method and with FreeSurfer were 162 ± 8 mL and 168 ± 9 mL, respectively. The tissue volumes measured by use of FreeSurfer were...
consistent larger in each subject, with a mean percent difference of 3.6 ± 1.1% (P = .005). The mean and SD of the spatial overlap were 0.945 ± 0.004. Images illustrating the tissue segmentation by the 2 methods are shown in Fig 6A–B, respectively. The corresponding PCF crowdedness indexes were 0.826 ± 0.012 and 0.856 ± 0.017, respectively. Because FreeSurfer does not provide the PCF volume, the PCF volume obtained by the proposed method was used to estimate the PCF crowdedness obtained by use of the 2 methods.

None of the 6 linear PCF measures were significantly associated with the PCF volume. Five of the linear measures correlated positively with the PCF volume with the following corresponding Pearson correlation coefficients: supraocciput length (r = 0.30, P = .31), length of cerebellum (r = 0.37, P = .20), clivus (r = 0.32, P = .27), Twining line (r = 0.30, P = .30), and length of body of the 2 methods.

The lack of a reliable automated method for PCF volumetry by use of MR imaging, however, limits the clinical use of these PCF markers. Advanced automated methods for brain parcellation have matured in recent years and are becoming more widely used.16,17 This work represents adaptation of established brain parcellation techniques tailored toward PCF volumetry in CMI. The proposed atlas-guided PCF segmentation method is enhanced by the creation of a CMI-specific reference atlas that captures the altered PCF morphology associated with CMI. An excellent agreement between the proposed automated method and manual segmentation by an expert observer is evident by the small relative percentage difference of −0.3 ± 1.9% and the very high mean Dice coefficient of 0.96. The delineation of the PCF obtained by use of the proposed automated method highly agrees with the manual delineation in terms of accuracy and spatial overlap in patients with CMI. Furthermore, a high degree of repeatability is evident from the small absolute percentage difference of 0.6 ± 0.2% found by use of quantification of the repeated scans in 3 healthy subjects. The automated volume measurement of PCF is minimally affected by the normal variability in patient positioning in the MR imaging scanner.

The mean PCF volume measurement obtained in our small cohort of adult patients with CMI (196 ± 8.7 mL) tends to be larger than previously reported CT and MR-based measurements of 186 mL by Nishikawa et al.11 174 ± 25 mL by Noudel et al.10 and 166 ± 8 mL by Milhorat et al.7 The bias in the mean volume measurements may be attributed to the differences in the modalities and the possible differences in the segmentation protocols, particularly how the PCF boundaries were defined. Another contributing factor may be related to the difference in the sampling resolution of the volumetric data. In contrast to isotropic 1-mm 3D imaging used in this work, previous reports used 2D-based imaging with thicker sections for the volumetric measurements that can lead to measurement errors caused by large partial volume effect. In addition, the limited number of subjects used in this study to validate the proposed automated method against manual segmentation may not be representative of a CMI population in terms of PCF volume.

The tonsillar herniation in CMI has been attributed to overcrowding of the PCF as a result of a small PCF and normally developed brain tissue volume.7,10,11 Therefore, in addition to PCF volume measurement, accurate quantification of brain tissue volume is also critical. Our measurement of mean PCF tissue volume of 162.1 ± 8.2 also tends to be slightly larger than previously reported values of 156 mL by Nishikawa et al.11 and 151.8 ± 3.1 mL by Milhorat et al.7 However, the measurement of crowdedness, the ratio of PCF tissue volume to PCF volume of 0.826 ± 0.012, is in good agreement with the mean value of 0.833 reported by Nishikawa et al.11

The comparison of the hindbrain tissue volume measurements between the proposed method and FreeSurfer revealed a statistically significant mean difference of 3.6 ± 1.1% (P = .005). The tissue volumes found through the use of FreeSurfer were consistently larger than volumes obtained by using the proposed method. As demonstrated in Fig 6, this difference is the result of

<table>
<thead>
<tr>
<th>Patient</th>
<th>PCF Tissue Volume (mL) (Proposed Method)</th>
<th>PCF Tissue Volume (mL) (FreeSurfer)</th>
<th>Percentage Volume Difference</th>
<th>Dice Coefficient</th>
<th>Crowdedness Index (Proposed Method)</th>
<th>Crowdedness Index (FreeSurfer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162.8</td>
<td>169.4</td>
<td>4.1%</td>
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<td>0.842</td>
<td>0.876</td>
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<td>172.1</td>
<td>181.6</td>
<td>5.5%</td>
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<td>0.828</td>
<td>0.874</td>
</tr>
<tr>
<td>3</td>
<td>151.9</td>
<td>156.2</td>
<td>2.8%</td>
<td>0.940</td>
<td>0.824</td>
<td>0.847</td>
</tr>
<tr>
<td>4</td>
<td>170.1</td>
<td>174.0</td>
<td>2.3%</td>
<td>0.948</td>
<td>0.831</td>
<td>0.850</td>
</tr>
<tr>
<td>5</td>
<td>153.9</td>
<td>158.6</td>
<td>3.1%</td>
<td>0.949</td>
<td>0.806</td>
<td>0.830</td>
</tr>
</tbody>
</table>

FIG 6. Outline of the PCF tissue masks (red) generated by use of the proposed method (A) and FreeSurfer (B) in 1 of the patients with CMI.

DISCUSSION

Quantification of the PCF volume and the degree of PCF crowdedness were shown to be beneficial for differential diagnosis of tonsillar herniation7,11 and for prediction of surgical outcome.10 The comparison of the hindbrain tissue volume measurements between the proposed method and FreeSurfer revealed a statistically significant mean difference of 3.6 ± 1.1% (P = .005). The tissue volumes found through the use of FreeSurfer were consistently larger than volumes obtained by using the proposed method. As demonstrated in Fig 6, this difference is the result of...
the exclusion of the tonsillar tissue volume that descends below the foramen magnum. This tissue is excluded because it is outside the PCF and thus does not contribute to the PCF overcrowding.

Assessment of the associations between PCF volumes and the linear PCF markers revealed that none of the 6 measures were significantly associated with the PCF volume. The 5 linear landmarks of the PCF were all modestly positively correlated with the PCF volume. The lack of significance can be explained in part by the small sample. The length of herniation negatively correlated with the PCF volume, which is expected when a normal size cerebellum is compressed inside an increasingly smaller PCF.

The reference atlas used to guide the segmentation was prepared by use of MR images from patients with CMI, all of whom had tonsillar herniation >5 mm. Therefore, this atlas may not be optimal for segmentation of healthy subjects because of morphological differences. However, for the purpose of reproducibility estimate, data from healthy subjects were used because repeated scans from patients with CMI were not available. Even with this limitation, an excellent reproducibility with an average difference of 0.6 ± 0.2% is obtained, reflecting the robustness of the proposed method.

CONCLUSIONS
The PCF volume and the degree of crowdedness can be reliably quantified in MR imaging data of patients with CMI by use of an atlas-based approach. Automatically delineated PCF compartments were similar in volume and spatial overlap with those delineated manually by an expert observer. These early results suggest that automated segmentation could substitute for manual delineation of the PCF, thereby advancing the use of PCF parcels for improved diagnosis and treatment decisions in CMI.

ACKNOWLEDGMENTS
The authors thank Lisa Kornse, BSN RN, for assistance with recruitment, Dr. Robert Quencer, MD, for supporting the Advanced Image Processing Lab, and the Evelyn F. McKnight Brain Institute for their support.

REFERENCES
Modelling of Spinal-Cord Fluid-Structure Interactions: Porous Effects

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Abstract:
Syringomyelia is a pathological neurological condition defined by the formation of one or more macroscopic fluid-filled cavities (syrinxes) in the spinal cord. The genesis of the fluid in a syrinx remains uncertain. A majority of evidence suggests that it is derived from cerebrospinal fluid (CSF) in the subarachnoid space, but the possibility that it arrives as interstitial fluid derived ultimately from capillary plasma has not been excluded. An existing axisymmetric fluid/structure-interaction numerical model (in ADINA) of the spinal cord and surrounding structures (Bertram 2010) had given results suggesting that a stenosis of the subarachnoid space might give rise to time-averaged pressure gradients such as to encourage CSF to move through the intervening pia and cord into an existing syrinx. To investigate whether such flows might occur, the model was therefore adapted to allow for porous fluid seepage between the subarachnoid space and a syrinx.

The model was implemented in the open-source software oomph-lib (Hazel & Heil 2006), using the poroelastic formulation reviewed by Simon (1992) to describe the two-phase system of a fluid permeating the pores of a solid matrix. Following the careful validation of the newly developed code against results obtained from the previous ADINA-based model, we are now able to assess the importance of hydrodynamically-induced seepage flows for the growth of a syrinx. Detailed results will be presented at the congress.

A grant from the Chiari & Syringomyelia Foundation is gratefully acknowledged.


Presentation Type (Complete): Invited Podium Presentation (no more than 2 total per speaker)
Track (Complete): Tissue Biomechanics
Session Details (Complete):
\begin{itemize}
  \item Session Chair : Bryn Martin, Francis Loth
  \item Session Title : Cerebrospinal Fluid Dynamics
\end{itemize}

Availability (Complete):
Status: Complete
CSF International Patient Registry Project
The randomized trial is one of the most powerful tools clinical researchers possess, a tool that enables them to evaluate the effectiveness of new (or established) therapies while accounting for the effects of unmeasured confounders and selection bias by indication. Randomized trials, especially huge megatrails, have transformed medical practice. Thanks to randomized trials, we no longer, for example, treat acute myocardial infarction with lidocaine and nitrates. Instead we use rapid revascularization, anticoagulants, and antiplatelet agents, and during long-term follow-up we routinely prescribe statins, beta-blockers, and angiotensin-converting–enzyme inhibitors. But the reputation of randomized trials has suffered of late, owing to reasonable concern about excess complexity, expense, and time required to recruit study participants, as well as inadequate representativeness. What good are trials if the results aren’t applicable to real-world patients and if, because of excessive expense, they can be used to answer only a tiny fraction of our important clinical questions?

One possible solution is to look to observational registries for answers. Over the past 20 to 30 years, a number of professional societies, government agencies, private corporations, and independent researchers have established high-quality registries that collect standardized data from patients seen in a variety of settings. In cardiovascular medicine, for example, registries in the United States and abroad have collected vast amounts of data from patients with acute coronary syndromes, stable coronary disease, and heart failure, as well as from patients with rare diseases such as hypertrophic cardiomyopathy and patients referred for surgery, percutaneous invasive procedures, and device implantation. Investigators and public health officials use registries to describe practice patterns and trends, to identify outliers, and to detect safety signals. They often use registries to assess comparative effectiveness, too, but are forced to admit that purely observational findings may not be internally valid owing to the absence of randomization.

As debates about comparative-effectiveness research have intensified over the past few years, we find ourselves in a kind of intellectual trap: yes, in theory we would like to conduct more randomized trials, but in practice they are too complex and difficult to apply to many clinical questions. And, yes, in theory we could answer many questions at
low cost with large-scale observational registries, but despite statistical advances, comparative observational registry studies are suspect because they lack the rigor of randomization.

Enter the registry-based randomized trial. With the Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE) trial, the results of which are now reported in the Journal (pages 1587–1597), a new paradigm has emerged that can potentially release us from the circular (and expensive) trap of the randomized-versus-registry debate. The TASTE investigators designed a large-scale trial to answer an important clinical question and carried it out at remarkably low cost by building on the platform of an already-existing high-quality observational registry. With this clever design, which leveraged clinical information that was already being gathered for the registry and for other preexisting databases, the investigators were able to quickly identify potential participants, to enroll thousands of patients in little time (see figure), to avoid filling out long case-report forms, to obtain accurate follow-up with minimal effort, and to report their findings, all for less than the amount of a typical modular R01 grant (i.e., a grant for research initiated by an individual investigator) from the National Institutes of Health. Their findings may well be broadly generalizable, since they included in the randomization process the majority of all patients treated for ST-segment-elevation myocardial infarction in the study area.

The registry-based randomized trial complements the strengths and addresses the weaknesses of the two most prominent types of comparative-effectiveness research. The trial is still a trial, a rigorous randomized experiment that isolates a causal link (or the absence of one) between a treatment and an outcome. Because the trial is inexpensive, investigators can enroll large numbers of patients, thus offering clinicians insights that are potentially based on a representative sample, a real-world population created from consecutively enrolled registry patients.

Despite this appeal, a number of fundamental questions must be addressed if we are to transform our clinical-research enterprise to give registry-based randomized trials, or other trials with highly efficient designs, a
prominent role. Will registry data (or data coming from other digital sources, such as electronic health records) be of high enough quality? Will too many data fields be missing? How will we balance efficacy versus effectiveness? Can we transition single registries from efficacy to effectiveness, making it possible to assess external validity much more expeditiously than we do now? What are the best populations or subpopulations to study? How will we approach concerns about privacy and informed consent (particularly in the context of trials that compare acceptable standards of care and use cluster-randomization methods)? Is blinding possible? Will researchers be able to obtain long-term follow-up or measure composite outcomes? How will we standardize and adjudicate certain outcomes? Can we assure representativeness, given that even within a registry there may be systematic differences between patients who are and are not eligible for randomization or between those who do or do not consent?

These are only some of the problems we will have to address. The TASTE trial was performed in Scandinavia, where the health care and information technology environments are markedly different from those elsewhere in the world. Can randomized registry trials be undertaken outside Scandinavia, in places where health care and clinical data are fragmented and of lower quality? Some American investigators are already using the approach (e.g., the Study of Access Site for Enhancement of Percutaneous Coronary Intervention for Women; NCT01406236). But even if we can perform many more randomized registry trials in the United States, we must recognize that the approach cannot solve all the problems we have with trials. For certain kinds of trials, such as metabolic efficacy studies that focus on complex physiologic and metabolic pathways hypothesized to respond to changes in diet or to experimental pharmacologic agents, current organizational structures would probably work much better with only minor modifications.

The randomized registry trial represents a disruptive technology, a technology that transforms existing standards, procedures, and cost structures. Will it be given serious consideration as a way to resolve the recognized limitations of current clinical-trial design? Theodore Roosevelt once said, "Do what you can, with what you have, where you are." Today we can no longer afford to undertake randomized effectiveness trials that cost tens or hundreds of millions of dollars. But today we also have registries and other powerful digital platforms. Today it may be possible to design and conduct megatrials with what we have: bigger data and smaller budgets. Yet we must also recognize and acknowledge the daunting challenges that diverse groups of researchers and stakeholders must overcome to get there.

The views expressed in this article are those of the authors and do not necessarily represent the official positions of the National Heart, Lung, and Blood Institute. Dr. Lauer is the National Institutes of Health representative on the Methodology Committee of the Patient-Centered Outcomes Research Institute (PCORI); none of the views expressed here represent those of PCORI or its Methodology Committee.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From the Office of the Director, Division of Cardiovascular Sciences, the National Heart, Lung, and Blood Institute, Bethesda, MD (M.S.L.); and the Mathematics and Statistics Department, Boston University, and the Harvard Clinical Research Institute — both in Boston (R.B.D.).

This article was published on September 1, 2013, at NEJM.org.


DOI: 10.1056/NEJMep1301102
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Sub-Group Agenda

I. Introduction
   a) Self-introduction
   b) Ask for email contacts to be sent to chair

II. Goal - Brief restatement of purpose

III. Review output documents of sub-group
   a) CDE structure appropriate for subgroup with example
   b) CRF inclusion

IV. Discussion of sub-group questions
   a) What should be included?
   b) How can it best be broken down?

V. Planned CDE process
   a) Division of the topic into groups
   b) Division of group into working teams/individuals
   c) Assignment of areas for CDE creation, CRF choice

VI. Milestones
   a) Establish mechanism and times of teleconferences
   b) Timeline for completion of draft
   c) Future general meetings
Chiari Malformation Project Proposal
Chiari and Syringomyelia Foundation

Chiari Malformation has been generally recognized as a disorder of the cervical-medullary junction that consists of crowding in the vicinity of the foramen magnum that may result in neurological symptoms. Because of the complexity of this region, the Chiari Malformation may present with a variety of symptoms including headache or other pain, cranial nerve dysfunction or extremity deficits based on cerebellar, brainstem or spinal cord compression. The variable presentation results in a large and variable differential diagnosis and great potential for misdiagnosis. A wide variety of misdiagnoses and long delay in diagnosis, averaging over 6 years, has been observed in clinical practice [1].

The most common Chiari symptom is headache, estimated to occur in approximately 80% of symptomatic cases [1]. National surveys have suggested a substantial disability attributable to migraine and other severe headaches with both physical and mental co-morbidity associated, making headache a major public health concern [2]. Given the prevalence of debilitating and refractory headaches, identifying a specific treatable cause in even a small fraction can be very significant. The accurate identification of those experiencing headache resulting from Chiari malformation, as opposed to another headache etiology, will have the potential to diagnose and cure those with headaches secondary to Chiari as well as reduce the risk of unnecessary and unhelpful surgery for those experiencing headaches from other etiologies.

While the number of people suffering headaches is immense, the number of anatomically defined Chiari patients identified is also growing. In the past decade [3-6], a significant increase in MR imaging has resulted in an increase in the radiological diagnosis of Chiari I malformation and a corresponding increase in patients radiologically identified who had questionable or no symptoms [7,8]. Unfortunately there is significant uncertainty as to the appropriate treatment of this anatomical finding in a variable clinical context [8]. Untreated symptomatic Chiari as well as secondary syrinx can cause severe damage to the brainstem and spinal cord. Untreated Chiari can result in long-term pain, neurological deficit and death. However, Chiari treatment often includes an invasive and expensive surgery with variable success. The large number of patients with anatomical Chiari and the large number with Chiari-attributed, but nonspecific, symptoms make accuracy of diagnosis difficult but of critical importance.

While the consequences of failure to recognize and surgically treat this disorder can be severe, the morbidity and cost of overtreatment with surgical decompression can be even more consequential. Anatomically, Chiari has an estimated incidence of approximately 1, 2 and 3% in adult males, females and children, respectively [9,10,11]. In one study, for example, this estimate was based on a tonsillar decent of >5mm in 14,000 MRI's obtained for any reason [10]. Surgery was performed on 35% of these discovered Chiari patients. Although results from all MRI’s done at an institution likely overestimates general population prevalence when applied to the general population (300 million, including 63 million children), over 5 million newly discovered anatomical abnormalities may potentially be observed. Based on the above estimates the potential perceived surgical need using current practice may swell to over 1-2 million cases. In contrast, many clinicians suspect the actual number of surgical candidates to be significantly lower, with prevalence estimates as low as 200,000 people (.06%) [12]. Thus, if current practice is applied to these anatomically identified cases, an unacceptably high rate of surgery would likely result.

In addition to a better understanding of surgical criteria in Chiari Malformation, continuing analysis of the incidence of other factors contributing to surgical failure and complications is needed. The technique of surgical decompression and presence of other factors such as pseudomeningocele likely play a role in outcome [13]. Procedures aimed at potential secondary causes of cerebellar herniation such as hydrocephalus, hereditary
connective tissue disorders, craniocervical instability, basilar invagination, tethered cord syndrome, and intracranial hypotension and hypertension may play a role that is as yet not well defined [14,15].

For reasons described above, without improved knowledge of the natural history, surgical criteria and outcome from specific surgical techniques, the number of operations (and re-operations) is likely to continue to increase. Currently 11,000 operations are performed in the US each year [7] at a cost of approximately $20-33,850 per surgical decompression [16, 17]. However, a potential increase in unnecessary surgery cost is a fraction of the additional cost incurred by the hospitalization, morbidity, and financial loss incurred by the sum of undertreated or overtreated Chiari patients.

As demonstrated in surveys of the neurosurgical community there is considerable disagreement and uncertainty about the treatment of Chiari [8]. Improved patient selection criteria and management based on knowledge about natural history and surgical outcomes would have a meaningful impact on the US population’s physical and economic health. The health and financial benefit of reduced morbidity care, increased independent function, reduced chronic medical and surgical treatment, and more focused diagnostic imaging would be significant if critical questions about the status of national Chiari care were addressed. Obtaining answers to these questions, aimed at improved medical effectiveness and efficiency, is the ultimate goal of the collaborative data being sought through this project.

Critical questions such as those below have generally been beyond the reach of small single institution studies:

1) What is the incidence and natural history of Chiari malformation with and without syringomyelia? Can we identify early those with high risk of clinical progression?
2) What are the causes and associated comorbidities (genetic, developmental and acquired) of the anatomical abnormalities observed?
3) What are the key anatomical findings which define Chiari malformation and which generate symptoms and neurological deficit?
4) What are the symptoms of Chiari malformation, their cause and response to treatment? How can they be differentiated from those resulting from other diseases?
5) How is Chiari malformation currently diagnosed and treated around the country? What is the effectiveness of these treatments in relieving patient suffering and morbidity? How do we define successful surgical treatment?

Goal:

Decrease morbidity and public health and social cost burden of Chiari malformation disorders through a significant increase in clinical information facilitated by the development of Common Data Elements (CDEs) and allowing the collection unprecedented multicenter Chiari data and the subsequent development of an international Chiari database. Through this process critical questions related to Chiari malformation can be addressed in a way not possible with the current Chiari research structure. Indeed, the registry-based randomized trial complements the strengths and addresses the weaknesses of the two most prominent types of comparative-effectiveness research [18].

Strategic aims:

1) Develop a Common Data Element Library, Clinical Research Forms, and accepted metrics and scales.
   Process:
   a. Organizational meetings
   b. Sub-Committee formation for CDE development
   c. CDE output per current NIH standard, through NIH collaboration
2) Identify current diagnostic and treatment practices and costs to target areas of needed improvement
3) Facilitate collaborative research of critical questions through development of a database
4) Facilitate international and multidisciplinary clinician and researcher involvement in Chiari Malformation research through committee involvement, seminar attendance and collaborative research.

The Role of the Chiari and Syringomyelia Foundation:

The Chiari and Syringomyelia Foundation (CSF) is a non-profit group with a national Scientific and Educational Advisory Board and regional chapters supporting education and research. Its stated mission is “To advance knowledge through research and educate the medical, allied science and lay community about Chiari Malformation, syringomyelia and related disorders.” The CSF, with an existent board of nationally and internationally interested clinicians and scientists, is in a unique position to leverage clinical research by supporting the integration of multi-institutional databases and studies. **We see the program of Common Database Element development as a fundamental first step in this process.**

Work to date:

1) The Database group of the CSF consists of eight CSF members, specialists and CSF staff who are tasked with developing a strategy to support clinical research through the development of a Chiari Registry and database. Members: Allison Ashley-Koch, Ulrich Batzdorf, Roger Kula, David Limbrick, Mark Luciano, Cormac Maher, Dorothy Poppe, Brandon Rocque.
2) Through regular Teleconference meetings the group has planned meetings and invited world and medical and industry for participation in the Sub-committees of the CDE process. A first round of national and international invitations has been sent to clinicians, researchers and industry. The specific committee acceptances to date are attached.
3) Meetings planned:
   a. April 5th: an initial meeting of the proposed CDE project
   b. Teleconference sub-committee meetings: TBA on individual group basis
   c. October 17, 2014 - Boston, MA: full CDE group meeting for initial report assembling Chiari CDE components
References:


CDE QUESTIONS – TREATMENT SUBGROUP

1. Indications for surgery: symptoms: specify
   Imaging findings: tonsil shape
   tonsil descent in mm
   medullary beak
   reversed vallecula
   compression on axials at foramen magnum

2. Size of Craniectomy
   Determined by: Measurement from MRI
   “Standard” measurement, i.e. we always do...
   Confirmed by measurement: actual measurement width, distance from foramen magnum in mm

3. Laminectomy
   C 1
   C 2
   other

4. Unusual appearance of dura

5. Ultrasound: Yes/no

6. Dura opening: Yes/No
   Full thickness/Outer layer only
   Unusual aspects of dura: hemorrhagic, scarred

7. Arachnoid opening: Yes/No
   Arachnoid excised: Yes/No
   Arachnoid strands divided: Yes/No
   Arachnoid retracted against dura for joint arachn. & dura closure

8. Tonsils: Retracted: Yes/No
   Reduced in volume: bipolar
   sub-pial resection: with/without pial closure
   limitations imposed by vessels, i.e. PICA, unilat/bilat
   Choroid plexus (IVth ventricle) visualized: yes/no

9. Fourth Ventricle: shunt/no shunt
   obex plug: yes/no
10. Duraplasty: No
   Yes: autologous pericranium
       biologic membrane, i.e. bovine pericardium
       synthetic: specify brand
       combination of any of the above
   Suture material
       Prolene – size
       Other
   Valsalva with closure: Yes/No
       Once/twice
       Wound filled/not filled

11. Cranioplasty: Yes/No
   Brand name
   Dural tack-ups: yes/no

12. Complications
    Early: [time from index procedure]
        infection
        CSF leak
        wound breakdown
        meningitis
        neurological deficit increase
    Late: [time from index procedure]
        recurrent tonsillar descent
        pseudomeningocele
        dural ectasia
        persistent tonsillar descent
        persistent distended syrinx
        cranio-cervical instability
        neurological deficit increase

13. Treatment Failures
    Persistent Symptoms
Stratified Whole Genome Linkage Analysis of Chiari Type I Malformation Implicates Known Klippel-Feil Syndrome Genes as Putative Disease Candidates

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Abstract

Chiari Type I Malformation (CMI) is characterized by displacement of the cerebellar tonsils below the base of the skull, resulting in significant neurologic morbidity. Although multiple lines of evidence support a genetic contribution to disease, no genes have been identified. We therefore conducted the largest whole genome linkage screen to date using 367 individuals from 66 families with at least two individuals presenting with nonsyndromic CMI with or without syringomyelia. Initial findings across all 66 families showed minimal evidence for linkage due to suspected genetic heterogeneity. In order to improve power to localize susceptibility genes, stratified linkage analyses were performed using clinical criteria to differentiate families based on etiologic factors. Families were stratified on the presence or absence of clinical features associated with connective tissue disorders (CTDs) since CMI and CTDs frequently co-occur and it has been proposed that CMI patients with CTDs represent a distinct class of patients with a different underlying disease mechanism. Stratified linkage analyses resulted in a marked increase in evidence of linkage to multiple genomic regions consistent with reduced genetic heterogeneity. Of particular interest were two regions (Chr8, Max LOD = 3.04; Chr12, Max LOD = 2.09) identified within the subset of “CTD-negative” families, both of which harbor growth differentiation factors (GDF6, GDF3) implicated in the development of Klippel-Feil syndrome (KFS). Interestingly, roughly 3–5% of CMI patients are diagnosed with KFS. In order to investigate the possibility that CMI and KFS are allelic, GDF3 and GDF6 were sequenced leading to the identification of a previously known KFS missense mutation and potential regulatory variants in GDF6. This study has demonstrated the value of reducing genetic heterogeneity by clinical stratification implicating several convincing biological candidates and further supporting the hypothesis that multiple, distinct mechanisms are responsible for CMI.


Editor: Ralf Krahe, University of Texas MD Anderson Cancer Center, United States of America

Received January 12, 2013; Accepted March 11, 2013; Published April 19, 2013

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Competing Interests: 1) Financial: An invention disclosure form (IDF) has been submitted for the invention titled “Genes Involved in Chiari Type I Malformation”. It was received by the Duke Office of Licensing and Ventures and assigned the IDF #3930 (CAM, SGG, and AAK), and 2) Professional: Dr. Allison Ashley-Koch is chair of the Chiari and Syringomyelia Foundation (CSF) scientific, education, and advisory board. CSF provided partial funding for this study, as well as salary support for Ms. Christina Markunas. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.
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† These authors contributed equally to this work.

Introduction

Chiari Type I Malformation (CMI) is characterized by displacement of the cerebellar tonsils below the base of the skull and occurs with an estimated prevalence of less than one percent in the United States [1,2]. Although magnetic resonance imaging (MRI) is considered the gold standard for diagnosis, no universally accepted diagnostic criteria exist. Patients are usually considered affected if one cerebellar tonsil is herniated 5 mm or more [3] or both tonsils are herniated 3 mm or more [4]. CMI patients exhibit a wide range of neurologic symptoms, including headaches, dizziness, difficulty sleeping, numbness/tingling of an upper extremity, fatigue, nausea, shortness of breath, blurred vision, among others [5]. Currently, the only treatment to alleviate symptoms for CMI is suboccipital decompression surgery to both expand the cranial base and re-establish normal cerebrospinal fluid (CSF) flow.

Although multiple mechanisms have been proposed for cerebellar tonsillar herniation, including cranial constriction, cranial settling, spinal cord tethering, intracranial hypertension, and intraspinal hypotension [6], “classical” CMI is generally hypothesized to occur through the “cranial constriction” mechanism. More specifically, “classical” CMI is thought to be caused by an underdeveloped occipital bone, resulting in a posterior fossa (PF) which is too small and shallow to accommodate the normal sized cerebellum [7,8]. Herniation of the cerebellar tonsils and an upward shift of the tentorium are thought to occur secondarily [8]. In addition to the “cranial constriction” mechanism, accumulating evidence supports an association between connective tissue disorders (CTDs) and some occurrences of CMI [9]. Importantly,
CMI patients diagnosed with CTDs may represent a distinct class of patients that can be grouped under the “cranial settling” mechanism where both the occipital bone and posterior cranial fossa volume are normal in size but occipitoatlantoaxial joint instability exists [6].

While no disease gene has been identified for CMI to date, several lines of evidence support a genetic contribution to disease in at least a subset of nonsyndromic cases. These include twin studies [1,10,11,12,13,14,15,16], familial clustering [10,14,15,17,18,19,20,21,22,23,24,25,26,27,28,29,30], and cosegregation with known genetic syndromes or conditions commonly found as part of a genetic syndrome, including Ehlers-Danlos syndrome [9,31,32,33], Marfan syndrome [9,34,35,36], Klippel-Feil syndrome [23,37,38,39,40,41,42,43,44,45,46,47,48], growth hormone deficiency [45,46,49,50,51,52,53,54,55], craniosynostosis [56,57], and Neurofibromatosis type 1 [58,59]. Furthermore, in a study conducted by Milhorat and colleagues, it was reported that out of a cohort of 364 symptomatic patients, 43 (12%) had at least one close relative with CMI with or without syringomyelia or idiopathic syringomyelia [23]. Additionally, 72 patients (20%) were reported as having at least one close relative with a similar symptomology without an official CMI diagnosis. Despite evidence for a genetic component, genetic studies for CMI have been limited. Ascertaining for family studies has been hindered due to a relatively rare disease prevalence together with the small proportion of cases that are familial [23]. In addition, the ability to obtain MRIs on a large series of individuals for diagnostic purposes and lack of consistent disease criteria has led to increased phenotypic variability across patients resulting in phenotyping challenges. Only one whole genome linkage screen, but no genome wide association studies, has been published for CMI. Using 23 Caucasian multiplex families containing 67 sampled individuals affected with CMI with or without syringomyelia, Boyles, et al. conducted a whole genome linkage screen and identified significant evidence for linkage to regions on chromosomes 9 and 15 [17]. While this study took an important first step in trying to elucidate the genetic basis of CMI, the genetics of CMI is still very much unknown. Our limited understanding of the biological mechanism, lack of consistent diagnostic criteria, and complex etiology pose exciting challenges for studying the genetics of CMI.

One major challenge is the variability of clinical presentation within the CMI patient population. This clinical heterogeneity presents as differences with respect to the pattern and severity of symptoms, response to surgery, presence of associated conditions, age of onset, and the extent of tonsillar herniation. As CMI is thought to be influenced by multiple genetic and environmental factors, this clinical heterogeneity likely reflects in part an underlying genetic heterogeneity. While this can have substantial implications during the design stage of a genetic study, the selection of families that are genetically homogeneous is not straightforward. One approach is to stratify families using clinical features that may identify groups of families that share similar genetic risk factors. In other words, reducing phenotypic variability may lead to a reduction in genetic variability. Although the pool of candidate clinical features to use for stratification can be quite large, previous clinical associations observed with the disorder provide some insight into which features to select.

To address these issues, we performed the largest whole genome linkage screen to date using 367 individuals from 60 nonsyndromic CMI multiplex families. Based on the limited evidence for linkage using the complete collection of families, we performed a stratified whole genome linkage analysis using the presence or absence of CTD related conditions and successfully identified putative CMI susceptibility genes in the genetically more homogeneous strata.

**Materials and Methods**

**Ethics Statement**

All participating family members provided written informed consent for this study. If participants were minors, written consent was obtained from a parent or legal guardian for participants younger than 18 years of age. Participants between 12 and 17 years of age were asked to provide written assent. Written informed consent and assent, when applicable, were obtained by approved clinical staff. Consent forms were either discussed in person or were mailed and then discussed over the telephone. All participant interactions were logged in Progeny 8 (Delray Beach, FL), our clinical data collection software program. The original signed consent is maintained by the study and a copy was provided to participants. The consent form, procedure described above, and this study were specifically approved by the institutional review board of Duke University Medical Center.

**Study Population**

Participants were ascertained across the United States primarily through self-referral in response to advertisements on the web (e.g. Duke Center for Human Genetics and GeneTests), mailings and/or presentations to patient support groups and physician referral. Families were enrolled in the current study if at least two sampled individuals were diagnosed with CMI with or without syringomyelia. Exclusion criteria included the following: 1) families with a positive family history of a known genetic syndrome (e.g. Ehlers-Danlos syndrome, Marfan syndrome, Klippel-Feil syndrome, Crouzon syndrome, Neurofibromatosis), 2) family history of spina bifida or tethered cord syndrome, and 3) individuals thought to have a secondary form of CMI, such as occurring due to a brain tumor. Although syndromic families formally diagnosed with hereditary CTDs were excluded from our genetic screen, many family members exhibited conditions such as hypermobility, mitral valve prolapse and scoliosis which are often associated with CTDs as described in further detail below. Blood samples were collected from affected individuals and all available connecting family members, regardless of affection status. Additionally, study participants completed a family and medical history telephone interview, responded to a detailed clinical questionnaire, and submitted release forms for medical records and pre-surgical MRIs. When possible, a diagnosis of CMI was determined based on MRI measurements in which affection status was defined as cerebellar tonsillar herniation of 3 mm or more for both tonsils or herniation of 5 mm or more for either tonsil (refer to Table 1 for MRI availability). MRI measurements were taken from pre-surgical T1-weighted brain MRIs. Herniation of the left and right tonsils was measured linearly from the tip of the cerebellar tonsils perpendicularly to the foramen magnum on a sagittal image to the left and right of the midline, respectively. All measurements were reviewed by a board certified neuroradiologist (D.E.). In the event that appropriate pre-surgical MRIs were not available, affection status was based on medical records or patient report when that was the only source available. Detailed population characteristics are provided in Table 1.

**Genotyping and Quality Control**

Blood samples were collected from study participants in EDTA tubes and DNA was extracted using the AutoPure LS® DNA extraction kit with Puregene® system reagents (Qiagen, Valencia, CA). A small amount of DNA (0.5 µg) was run on a 0.8% agarose
gel in order to assess quality and each sample was quantified using the Nanodrop (Wilmington, DE). In total, 436 individuals from 75 families were genotyped using Illumina Human610-Quad Bead-Chips (San Diego, CA) per the manufacturer’s instructions and chips were scanned using the Illumina iScan system (San Diego, CA). Due to the duration of ascertainment for this study, genotyping was performed in two separate batches (Batch 1: 234 individuals from 40 families; Batch 2: 202 individuals from 41 families). In addition to samples from study participants, replicate samples were included across sample plates and checked for mismatches. Specifically, two CMI family (1 male, 1 female) and two Centre d’Etude du Polymorphism Humain (CEPH) (1 male, 1 female) samples were included across three 96-well sample plates per batch in an alternating pattern.

Quality control (QC) procedures were performed to ensure high quality data were used for analysis. Initial quality assessment was

Table 1. Population characteristics.

<table>
<thead>
<tr>
<th>Description</th>
<th>No. Individuals</th>
<th>No. Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>367a</td>
<td>66</td>
</tr>
<tr>
<td>Number of affected individuals/family</td>
<td>2.77 ± 0.99 [2–6]b</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>223</td>
<td>65</td>
</tr>
<tr>
<td>Male</td>
<td>144</td>
<td>61</td>
</tr>
<tr>
<td>CMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected</td>
<td>183</td>
<td>66</td>
</tr>
<tr>
<td>Female</td>
<td>124</td>
<td>60</td>
</tr>
<tr>
<td>Male</td>
<td>59</td>
<td>44</td>
</tr>
<tr>
<td>Unaffected/Uncertain</td>
<td>184</td>
<td>61</td>
</tr>
<tr>
<td>Female</td>
<td>99</td>
<td>51</td>
</tr>
<tr>
<td>Male</td>
<td>85</td>
<td>53</td>
</tr>
<tr>
<td>Syringomyelia</td>
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<td></td>
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<tr>
<td>Affected</td>
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<td>19</td>
</tr>
<tr>
<td>CMI-Unaffected/Uncertain</td>
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<td>3</td>
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<tr>
<td>Female</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Unaffected/Uncertain</td>
<td>317</td>
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<tr>
<td>CMI-Affected</td>
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<tr>
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<tr>
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<tr>
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<td>Male</td>
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<td>53</td>
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<td>91</td>
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<tr>
<td>CMI-Affected</td>
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<td>52</td>
</tr>
<tr>
<td>CMI-Unaffected/Uncertain</td>
<td>153</td>
<td>54</td>
</tr>
</tbody>
</table>

*Only considered genotyped individuals after exclusions were applied (See Methods section for details).

bMean ± standard deviation [range].

Abbreviations: CMI: Chiari Malformation Type I; No.: number.

doi:10.1371/journal.pone.0061521.t001
performed separately for each batch using the Illumina Geno-
meStudio genotyping module (San Diego, CA). Single nucleotide
polymorphism (SNP) data (N = 585497 combined across batches 1
and 2) quality were further assessed using PLINK v1.07 [60] to
detect deviations from Hardy-Weinberg equilibrium (HWE;
calculated using unaffected founders), estimate minor allele
frequency (MAF; calculated using unaffected founders), and
detect Mendelian errors (Parent-Parent-Child (P-P-C); Parent-
Child (P-C)) errors were identified separately using custom scripts
since PLINK does not examine trios with missing parents.
Additional sample quality checks in PLINK included estimating
pairwise identity by descent (IBD) in order to verify known
relationships and check for cryptic relatedness (−genome; markers
were pruned first), identifying Mendelian errors as described
previously, calculating inbreeding coefficients (−het; markers were
pruned first), performing a multidimensional scaling analysis in
order to detect population stratification as different ethnicities
could alter MAF estimates thus affecting the linkage analysis (1
individual per family used; −cluster −ppc 1e−4−mds−plot 2; markers
were pruned first), and checking for sex discrepancies (−check-sex).

Whole Genome Linkage Analysis

Power for the whole genome linkage study was determined using
SIMLINK [61]. Family structures, disease and sample
statuses were based on the CMI multiplex linkage families used in
the screen and provided as input for the simulations (N_{rpt} = 1,000).
Additional model parameters used for the simulations included:
disease MAF of 0.001, marker MAF of 0.30, and
an affecteds-only, low penetrance function (0, 0.001, 0.001).

All linkage analyses were performed using MERLIN 1.1.2 and
MINX (MERLIN in X) [62] and allele frequencies were estimated
using founders only for all analyses subsequently described. Since
the underlying genetic model for CMI is unknown, both
parametric (model dependent) and nonparametric (model free)
linkage analyses were performed. For the parametric linkage
analysis, an “affecteds only” low penetrance function was used (0,
0.001, 0.001) and a rare disease allele frequency of 0.001 was
assumed. We performed an “affecteds-only” analysis because
affected/unknown individuals will only contribute genotypic
information, while affected individuals will contribute both
phenotypic and genotypic information to the analysis. This
approach protects against misclassification of non-penetrant
individuals within the families. In addition to the standard LOD
score analysis, MERLIN also provides estimates of the proportion
of linked families (2) and the maximum heterogeneity LOD score
(HLOG) which was used to detect linkage allowing for heteroge-
nity for the parametric analysis [62]. For the nonparametric
linkage (NPL) analysis, the S_{all} scoring function was used which
assesses IBD sharing across subsets of affected individuals [63]. In
addition, both the Kong and Cox linear and exponential model
were applied in order to evaluate statistical significance [64].

For both the parametric and nonparametric linkage analysis,
two-point and multipoint analyses were performed. In order to
maintain the correct type I error rate when conducting a multi-
point analysis in families when one or both parents are missing,
the option, “−rsq”, in MERLIN was implemented which allows for
the modeling of inter-marker linkage disequilibrium (LD) between
SNPs [62]. An r^2 threshold of 0.16 [65] was selected to group
SNPs into clusters.

Prior to whole genome linkage analysis, MERLIN’s error
detection option was used to identify possible genotyping errors,
such as unlikely double recombinants [62]. All genotypes flagged
as potentially problematic were set as missing for the linkage
analysis.

Stratified Whole Genome Linkage Analysis

Families (N = 66) were stratified based on medical record
documentation or self-reported family history of any of the
following CTD related conditions: hypermobility (N = 4), kyphosis
(N = 2), mitral valve prolapse (N = 9), pectus
excavatum (N = 1), scoliosis (N = 15), orthostatic hypotension
(N = 1), supraventricular tachycardia (N = 2), heart valve disease
(N = 12), and/or heart murmur (N = 6). In total, 34 families were
grouped as “CTD-positive” and the remaining 32 families were
“CTD-negative”. CTD-positive families had a significant history
for one (47.1%), two (32.4%), three (14.7%), or five (5.9%) of
the CTD-related conditions described above.

Permutation Tests

A series of permutation tests were performed using custom
scripts in order to determine genome-wide and chromosome-wide
empirically derived significance levels for the stratified analyses
conditional on the prior evidence for linkage. This was used to
assess the relationship between the increased evidence for linkage
and clinical criteria used to stratify families. For both the
parametric (two-point and multipoint) and nonparametric linear
and exponential model; two-point and multipoint analyses
the following was performed: 1) The dataset was randomly split in half
creating two datasets each containing 33 families, 2) Linkage
analyses were conducted using MERLIN 1.1.2 and MINX for the
X chromosome [62] as previously described in each set of families
separately, 3) For each analysis (N = 6), the maximum LOD score
was retained for each chromosome as well as genome-wide, and 4)
Steps 1 through 3 were repeated 500 times in order to construct an
empirical distribution (N_{total} = 1000).

Candidate Gene Sequencing

Candidate gene selection for de novo sequencing was based on results
from the CTD stratified whole genome linkage analysis described below. All affected individuals from any of the 66
linkage families that showed a positive family specific LOD score
for the peak marker on chromosome 8 (rs2446871) or chromo-
some 12 (rs10505755) were selected for Sanger sequencing of
growth differentiation factors, GDF6 and GDF3. In total, 96
affected individuals from 39 families and 75 affected individuals
from 28 families were initially screened for mutations in GDF6
and GDF3, respectively. Seventeen GDF6 primer sets were
designed to cover the exons (including intron-exon boundaries),
5’ and 3’ untranslated regions (UTR), as well as three intronic
regions with high conservation (UCSC genome browser: Placental
Mammal Basewise Conservation by PhyloP). Three GDF3 primer
sets were designed to cover exons (including intron-exon
boundaries) and 5’ and 3’ UTRs. Primer sequences, PCR
conditions and kits are described in detail (Tables S1–S2). PCR
amplicons and primers were sent off to Agencourt (Danvers, MA)
and GeneWiz (South Plainfield, NJ) for Sanger sequencing. SNPs,
as well as insertions and deletions (indels) were identified using
Sequencer 5.0 (Ann Arbor, MI) and all sequences were manually
inspected for each variant and indel called. Additionally, all
individuals were checked for sufficient sequencing coverage for
each amplicon. The nomenclature used to describe novel variants
was based on recommendations by den Dunnen and Antonarakis
[66]. Bi-directional sequencing in affected as well as unaffected
family members was performed in order to follow-up eight
identified variants that met subsequent criteria: 1) 1000 Genomes
European MAF <0.05 (Integrated Phase 1 Release v3), 2)
Identified in more than one affected individual (except two novel
variants that were identified within the same family), and 3) 1000
Genomes European MAF was less than the study population MAF
which was roughly estimated using all affected family members. Sequence data for novel variants were submitted to GenBank under accession numbers KC174775-KC174780.

Results

Genotyping Quality

Out of the 59232 SNPs genotyped on the Illumina Human610-Quad BeadChips (San Diego, CA), 7544 (1.3%) and 6835 (1.2%) SNPs were excluded from batches 1 and 2, respectively, due to call rates <98%, presence on chromosomes 24–26, high replicate error rate, as well as Illumina specific quality metrics including AB T Mean, AB R Mean, cluster separation, among others. Within each batch, replicate reproducibility rates exceeded 99.999% and all samples, except for one of the CEPH samples in batch 2, had a call rate >99%. Additional SNPs were excluded with Mendelian errors in >4% families (N = 220), MAF <0.05 (N = 66555), HWE p <0.001 (N = 275), identical physical location (Human genome build GRCh37/hg19; N = 2), no genetic distance available from deCODE, (N = 948), call present in only batch 1 (N = 2445), call present in only batch 2 (N = 2991), and identical genetic position (based on two decimal places; N = 290918). Genotyes for all SNPs showing non-Mendelian inheritance were set as missing for the entire family. A total of 221343 SNPs remained after filtering and were used to construct the two-point linkage map. From those remaining SNPs, 12056 were selected for use in the multipoint linkage analysis using criteria such as genetic distance in order to create an evenly spaced map and high MAF estimates resulting in increased marker heterozygosity (Mean distance (cM) between SNPs: 0.31±0.008; Mean MAF: 0.42±0.09). In addition to SNP exclusions, three individuals were excluded due to large genomic duplications and/or regions of loss of heterozygosity detected from log R ratio and B allele frequency plots in Illumina GenomeStudio. This ultimately resulted in a total loss of 14 individuals due to two families that were no longer useful for linkage analysis. After additional sample exclusions were applied, 367 individuals from 66 families remained for analysis. Detailed sample exclusions are provided, along with the multidimensional scaling analysis used to identify sample outliers (Table S3 and Figure S1).

Whole Genome Linkage Screen: Primary Analysis

SIMLINK [61] was used to estimate power for our whole genome linkage screen. Assuming homogeneity and a low recombination fraction (θ = 0.01), the probability of obtaining a LOD score exceeding 3 was 0.94 using all 66 families collectively suggesting that we had adequate power to conduct the whole genome screen. Following data quality assessment, both two-point and multipoint parametric and nonparametric linkage analyses were conducted. Initial findings across all 66 families showed minimal evidence for linkage, with no multipoint maximum LOD scores exceeding 2 although several two-point LOD scores exceeded 3 across the various models (See Table S4 for summary). Although no multipoint LOD scores exceeded 2, maximum multipoint LOD scores between 1.25 and 2 were found on 2q37.3 (Max LOD = 1.40, exponential model), 8q21.3–q22.2 (Max LOD = 1.38, linear model), 9p22.3–p21.3 (Max HLOD = 1.96, p = 0.28), 9q22.31–q22.33 (Max LOD = 1.32, linear model), 12p13.13–p13.2 (Max HLOD = 1.48, p = 0.25), and 16q21.33–q22.3 (Max HLOD = 1.78, p = 0.22). Based on the limited significance of these results, stratified analyses using clinical criteria were conducted in order to reduce potential genetic heterogeneity thus improving power to localize CMI susceptibility genes.

Stratified Whole Genome Linkage Screen

Families were stratified based on a family history of CTD related conditions and two-point and multipoint nonparametric and parametric whole genome linkage analyses were performed within the CTD-negative and CTD-positive group of families separately. Genome-wide results from the two-point analyses are shown in Figure 1 and the most significant two-point results are included in Table 2. As expected, different regions of the genome exhibit evidence for linkage depending on the subset of families examined. No two-point LOD scores under a linear model exceeded 3 within either family subset and were therefore not included in Figure 1 or Table 2. Maximum multipoint LOD scores exceeding 2 within either set of families are summarized in Figure 2 and Table 2. While no multipoint LOD scores exceeding 2 were previously obtained when all 66 families were analyzed collectively, multiple genomic regions now exhibit maximum LOD scores exceeding 2 and in one case exceeding 3 on chromosome 8. Notably, the most significant two-point LOD scores are found within the 1 LOD down supporting intervals for regions on chromosomes 8, 9, and 12 within the CTD-negative group of families and regions on chromosome 1 within the CTD-positive group of families (Table 2).

Permutation Tests

In order to assess the relationship between the CTD stratification criteria and evidence for linkage, both genome-wide (GW) and chromosome-wide (CW) empirical p-values were obtained for both multipoint and two-point analyses under the three linkage models. Although no marker met GW significance, the peak marker for 8q21.3–q22.1 had a GW empirical p-value of 0.07 with a highly significant CW empirical p-value of 0.008. Additionally, several markers from the two-point and multipoint analyses had CW empirical p-values less than 0.05 as shown in Table 2. It is important to note that the empirical p-values derived from the permutation tests are approximate due to the fact that these families are of different sizes and structures.

Candidate Gene Sequencing

Sanger sequencing was performed on all affected individuals from families with a positive LOD score for the linkage peak marker in the chromosome 8 or 12 linkage regions. The primary focus was on the most significant multipoint linkage peak found on chromosome 8 within the CTD-negative group of families (8q21.3–q22.1; Max LOD = 3.04, linear model). The 1 LOD down supporting interval contained 49 candidate RefSeq genes (Chr8:91334498–8960813, GRCh37/hg19). Of those, one of particular interest was Growth differentiation factor 6 (GDF6) which is a member of the bone morphogenetic protein (BMP) subfamily and has been previously associated with a wide range of phenotypes including ocular, such as microphthalmia and coloboma, as well as skeletal, such as Klippel-Feil syndrome (KFS) which is characterized by fusion of any two of the seven cervical vertebrae [67,68]. The candidate interval on chromosome 12p13.13–p13.2 (Max HLOD = 2.09, 1 LOD down interval: Chr12:7794736–12721298, GRCh37/hg19) identified within a clinically similar subset of families (CTD-negative) also harbored a growth differentiation factor (GDF3), mutations in which have been previously associated with KFS [69]. As CMI and KFS may be allelic disorders, both GDF6 and GDF3 were selected for candidate gene sequencing in order to identify mutations and/or rare variants that increase susceptibility for disease.
In total, 22 SNPs, 2 insertions, and 1 deletion were found in GDF6 and 3 SNPs were found in GDF3 (Table S5). Of these, 6 were novel and 12 were rare (1000 Genomes European MAF <0.05) in GDF6 and 1 was rare in GDF3. In order to validate and establish segregation for a subset of these variants, 8 variants (7 in GDF6 and 1 in GDF3) were selected for follow-up sequencing (Table 3; see Methods under the candidate gene sequencing section for selection criteria). Within this subset of rare and novel variants, complete sharing across affected family members was observed with only two of the variants: 1) Novel SNP, g.406+2780C>T and 2) rs140757891. Reduced penetrance was observed for all variants of interest, except for rs121909352 although this is likely due to the fact that DNA samples were not available for all family members. Of particular interest is the missense variant, rs121909352 (A249E), which is a heterozygous mutation previously identified in KFS patients [67,68] as well as patients with microphthalmia and coloboma [68,70,71]. Pedigrees showing segregation of this mutation with affection status are shown in Figure 3. Within family 9453, all individuals presenting with CMI and syringomyelia, except for individual 2002, were

![Figure 1. Whole genome two-point LOD scores obtained from stratified analysis.](http:// gettinggeneticsdone.blogspot.com/)

doi:10.1371/journal.pone.0061521.g001
heterozygous for the mutation. Individual 2002 was found to have increased homozygosity as determined by an F inbreeding coefficient $>4$ standard deviations away from the mean and had been previously removed from the linkage analysis. In addition, one individual presenting with a suspected Chiari Malformation Type 0 (CM0) in family 9453 was heterozygous for the mutation; a detailed clinical description of this individual has been provided previously [72]. CM0 patients present with syringomyelia without tonsillar herniation that improves following posterior fossa decompression surgery. In family 9476, only one individual diagnosed with CMI and syringomyelia was heterozygous for the mutation (Figure 3); one additional individual with CMI and syringomyelia (1004) and one individual with tonsillar ectopia (1001) did not have the mutation.

In addition, the two intronic variants (Novel SNP, g.406+2780C>T and rs140757891) that are shared across all affected family members are located within potential regulatory regions (Table 3). The novel intronic SNP (g.406+2780C>T) is located within a predicted regulatory region for the protein, Suppressor of zeste 12 homolog (SUZ12), based on chromatin

Figure 2. Two-point and multipoint LOD scores obtained from stratified analysis. Only chromosomes with a maximum multipoint LOD score $>2$ are shown. LOD score thresholds of 2 and 3 are indicated by the blue and red lines, respectively. Green points and lines represent LOD scores under a linear model, blue points and lines represent HLOD scores, and red points and black lines represent LOD scores under an exponential model. CTD-positive families: Chr1 (A), CTD-positive families: Chr9 (B), CTD-negative families: Chr8 (C), CTD-negative families: Chr9 (D), CTD-negative families: Chr12 (E), and CTD-negative families: Chr17 (F). Negative two-point and multipoint LOD scores are set to zero. Plots were created in R 2.15.0. doi:10.1371/journal.pone.0061521.g002
immunoprecipitation sequencing (ChIP-seq) data from the Encyclopedia of DNA Elements Consortium (ENCODE) (UCSC Genome browser: GRCh37/hg19 human assembly). The rare intronic SNP, rs140757891, is also located within a predicted regulatory region for SUZ12 as well as the GPA binding protein 2 (GATA2) based on ChIP-seq data from ENCODE (UCSC Genome browser: GRCh37/hg19 human assembly). In addition, rs140757891 is part of a CpG dinucleotide located within a predicted CpG island spanning 701 base pairs (UCSC Genome browser: GRCh37/hg19 human assembly). When the variant allele is present the guanine (G) becomes an adenine (A) (reverse strand). Segregation of these two intronic variants, along with two additional novel variants (g.18328T>G and g.15169-59T>A) found in one of these families are provided in Figure S2.

**Discussion**

In order to gain a better understanding of the genetic architecture of CMI, we conducted a whole genome linkage screen using a collection of 66 nonsyndromic families with at least two sampled individuals presenting with CMI with or without syringomyelia. It was hypothesized that the limited evidence for linkage across all 66 families collectively was due to genetic heterogeneity and may be associated with the phenotypic variability observed. Based on the co-occurrence of CMI and CTDs, families were stratified by CTD related conditions in order to identify phenotypically and potentially genetically more homogeneous groups of families for linkage analysis. Stratified analyses identified multiple genomic regions showing increased evidence for linkage consistent with reduced genetic heterogeneity across families as a result of the CTD related stratification criteria. Furthermore, several plausible disease genes were identified as discussed in detail below.

Prior to describing our most significant results, it is important to relate our findings to the only other whole genome linkage screen conducted to date which implicated regions on chromosomes 9 and 15 [17]. We only identified suggestive evidence for linkage to the region on chromosome 9 within our CTD-positive group of families. Importantly, 12/66 of our total families and 7/34 CTD-positive families overlap with the families used in the initial screen conducted by Boyles and colleagues; therefore, these results do not provide independent replication for this region. Lack of replication for chromosome 15 could be due to the use of: 1) different genotyping chips (Illumina Human610-Quad BeadChips versus Affymetrix 10K SNP Chip) and marker quality control procedures, 2) different linkage software packages (Merlin versus Allegro; e.g. different with respect to an error detection option and accounting for inter-marker LD) and genetic models (penetrance function and S scoring function), 3) additional families which are likely genetically heterogeneous, and/or 4) different analytical approaches (stratified analyses). While the original finding could be a false positive, it is equally possible that as additional families are collected and other approaches to reduce genetic heterogeneity are applied to the data this region may present again as a promising candidate genomic interval warranting follow-up.

While we presented linkage results within the subsets of both CTD-positive and CTD-negative families, the focus of the current paper has been on the CTD-negative families as these are thought to represent more "classical" CMI due to cranial constriction and

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**Table 2. Most significant two-point and multipoint LOD scores.**

<table>
<thead>
<tr>
<th>Family description</th>
<th>Linkage model</th>
<th>Location (markers)b</th>
<th>Two-point LODc</th>
<th>Emp p-value (CW/GW)d</th>
<th>Multipoint LODc</th>
<th>Emp p-value (CW/GW)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTD-positive</td>
<td>Parametric: dominant</td>
<td>18q22.1 (rs17079623, rs574539)</td>
<td>4.53</td>
<td>0.027/0.150</td>
<td>0.71</td>
<td>0.787/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1q23.2 (rs2165593, rs3862952)</td>
<td>4.42</td>
<td>0.053/0.208</td>
<td>1.63</td>
<td>0.131/0.834</td>
</tr>
<tr>
<td></td>
<td>NPL: exponential</td>
<td>7p15.3 (rs14766697, rs4719814)</td>
<td>4.46</td>
<td>0.025/0.553</td>
<td>0.57</td>
<td>0.601/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18q22.1 (rs17079623, rs2048329)</td>
<td>4.44</td>
<td>0.059/0.573</td>
<td>0.42</td>
<td>0.856/1</td>
</tr>
<tr>
<td></td>
<td>NPL: linear</td>
<td>1q23.2-q24.2 (rs10494477)</td>
<td>0.87</td>
<td>1/1</td>
<td>2.63</td>
<td>0.032/0.184</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1q23.2-q41 (rs3862952)</td>
<td>0.35</td>
<td>1/1</td>
<td>2.3</td>
<td>0.053/0.356</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9q21.31-q22.31 (rs10746837)</td>
<td>1.49</td>
<td>1/1</td>
<td>2.22</td>
<td>0.112/0.423</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9p22.3-p21.31 (rs2840790)</td>
<td>0.27</td>
<td>1/1</td>
<td>2.15</td>
<td>0.133/0.484</td>
</tr>
<tr>
<td>CTD-negative</td>
<td>Parametric: dominant</td>
<td>8q22.3 (rs12545537, rs544821)</td>
<td>3.72</td>
<td>0.156/0.871</td>
<td>0.01</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9p24.2 (rs2181829, rs7024139)</td>
<td>3.62</td>
<td>0.316/0.928</td>
<td>1.26</td>
<td>0.596/0.990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12p13.31-p13.2 (rs6488255)</td>
<td>0.63</td>
<td>1/1</td>
<td>2.09</td>
<td>0.066/0.439</td>
</tr>
<tr>
<td></td>
<td>NPL: exponential</td>
<td>8q22.1 (rs1597301, rs6989464)</td>
<td>4.69</td>
<td>0.031/0.394</td>
<td>1.73</td>
<td>0.066/0.768</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12p13.2 (rs7312834, rs2055334)</td>
<td>4.54</td>
<td>0.014/0.498</td>
<td>0.85</td>
<td>0.414/1</td>
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<td></td>
<td>17p12 (rs6502282)</td>
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<td>0.044/0.491</td>
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<td>NPL: linear</td>
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<td>2.04</td>
<td>0.008/0.070</td>
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<tr>
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<td></td>
<td>17p12-q11.1 (rs7406339)</td>
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<td>1/1</td>
<td>2.37</td>
<td>0.027/0.309</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9p24.3-p24.2 (rs1416621)</td>
<td>1.72</td>
<td>1/1</td>
<td>2.29</td>
<td>0.097/0.366</td>
</tr>
</tbody>
</table>

*aThe top two most significant two-point results within each model and family subset as well as any maximum multipoint LOD score exceeding 2 are included.

*bWhen two markers are listed, the first corresponds to the marker used for the two-point result shown. The second corresponds to the closest marker included in the multipoint analysis.

**Abbreviations:** CTD: connective tissue disorder, NPL: nonparametric linkage, LOD: logarithm of the odds, Emp: empirical, CW: chromosome-wide, GW: genome-wide, N/ A: not applicable.

doi:10.1371/journal.pone.0061521.t002
also resulted in the identification of the only genomic region with a maximum LOD score exceeding 3. The most significant of our findings implicated the growth differentiation factors, GDF6 and GDF3, both of which had been previously implicated in KFS [67,68,69] which is characterized by cervical vertebral fusion and may be associated with a wide range of conditions including renal abnormalities, cardiovascular abnormalities, orthopedic anomalies, pulmonary problems, deafness, and synkinesia [73]. Interestingly, roughly 3–5% of CMI patients are diagnosed with KFS [23,45], suggesting a shared genetic etiology between these disorders. Further, it has been proposed that KFS and CMI should be classified as post-otic neural crest syndromes, thus sharing a common cellular etiology [74]. Although the exact relationship between these disorders is unknown, one possibility is that CMI and KFS may be allelic disorders. In order to investigate this possibility, GDF3 and GDF6 were sequenced in a collection of CMI patients from our linkage families. While GDF3 still presents as an intriguing biological candidate and additional sequencing of potential regulatory elements may yield putative disease variants, no variants of obvious significance were identified in this study. However, several interesting variants were identified in GDF6. A previously identified KFS mutation, A249E (rs121909352), was found in two of our CMI families. The functional effect of this mutation has been determined previously in vitro. Asai-Coakwell and colleagues evaluated changes to bone morphogenetic protein (BMP) signaling by co-transfecting an expression construct with the A249E mutation and a Sex determining region Y-box 9 (SOX9)-responsive reporter gene into primary limb mesenchymal cells.
mature GDF6 protein expression was observed for the mutant as
genetic potential [68]. In addition, a 23% reduction in secreted
and assessed SOX-9 reporter activity [68]. Reduced activation of
encompass NF1 as well as other genes, including SUZ12 resulting
mutations in NF1 some harbor larger genomic deletions that
spots [79]. SUZ12 and NF1 are located within 560 kb of each
development of neurofibromas and the presence of cafe ´-au-lait
neurofibromatosis 1 (NF1), a disorder characterized by the
SUZ12 and central nervous system disorders may come from
Miro and colleagues suggest that an additional link between
tectum and only demonstrate partial clinical similarity with CMI,
an enlarged brainstem, and occipital cortical changes [79].

Additional variants of interest from our study include two
intronic GDF6 variants, rs140757891 and a novel SNP,
g.406+2780C>T. ChIP-seq data from a small number of cell
lines indicate that both variants are located within predicted
targets of SUZ12, a polycyto protein involved in epigenetic
silencing of developmental genes. Interestingly, haploinsufficient
SUZ12 mice exhibit cerebellar herniation, as well as spina bifida,
an enlarged brainstem, and occipital cortical changes [79].
Although these clinical features appear to be due to an enlarged
and only demonstrate partial clinical similarity with CMI,
Miro and colleagues suggest that an additional link between
SUZ12 and central nervous system disorders may come from
neurofibromatosis 1 (NF1), a disorder characterized by the
development of neurofibromas and the presence of cafe ´-au-lait
spots [79]. SUZ12 and NF1 are located within 560 kb of each
other on chromosome 17 and while most NF1 patients have point
mutations in NF1 some harbor larger genomic deletions that
encompass NF1 as well as other genes, including SUZ12 resulting
in a more severe clinical presentation [79]. Roughly 5% of CMI
patients present with NF1 [45] and it has been previously
suggested that these two disorders may share an underlying genetic
basis [58]. Remarkably, within the same group of families that
showed increased evidence for linkage to the region containing
GDF6 (CTD-negative) we also observed suggestive evidence for
linkage to 17p11.2–q11.2 (Max LOD = 2.37, CW emp p-val = 0.03)
which contains both SUZ12 and NF1 providing further support
for a potential role in disease development.

While encouraged by our findings, we acknowledge several
limitations of this study. First, because we enforced strict eligibility
criteria (exclusion of syndromic cases) and required families to
have multiple affected individuals, the total number of families
eligible for the study was low and likely contributed to reduced
power. However, despite the relatively small sample size, the
number of families examined was almost three times as large as the
collection of families used in the only other whole genome linkage
screen published to date [17]. Second, MRIs were not available for
all study participants thus misclassification of affection status
cannot be ruled out. Importantly, none of our analyses used
phenotype information from “unaffected” family members (i.e.
affects-only analysis), thus the greatest impact of potential
misclassification would be if individuals were incorrectly classified
as affected. Furthermore, clinical information used for the
stratified analysis was mostly ascertained through a general
medical interview upon enrollment in the study; therefore,
misclassification of families as CTD-positive or CTD-negative is
possible. Nevertheless, our data suggest that the increased evidence
for linkage observed for the stratified analysis based on CTD
related conditions is non-random (e.g. 8q21.3–q22.1: GW emp p-
val = 0.07, CW emp p-val = 0.008). This observation would seem
unlikely if a high degree of misclassification existed.

Future work will include functional follow-up of variants of
interest as well as sequencing GDF3 and GDF6 in a larger cohort
of sporadic and familial CMI cases. Furthermore, the distant
regulatory elements previously identified for GDF6 [80,81,82]
represent excellent candidate regions for future de novo variant

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>Location⁹</th>
<th>Variant ID⁹</th>
<th>Alleles²</th>
<th>Variant Class</th>
<th>CMI/1KG MAF*</th>
<th>All Affsf</th>
<th>Reduced Pen⁹</th>
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</thead>
<tbody>
<tr>
<td>GDF6</td>
<td>8</td>
<td>97154593</td>
<td>g.18328T&gt;G</td>
<td>T/G</td>
<td>3' UTR</td>
<td>0.005/NA</td>
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<td>Yes (1/2)</td>
</tr>
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<td>rs112542818</td>
<td>C/T</td>
<td>3' UTR</td>
<td>0.026/0.003</td>
<td>No (5/6)</td>
<td>Yes (2/4)</td>
</tr>
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<td>97157223</td>
<td>rs148861809</td>
<td>C/G</td>
<td>Coding-syn</td>
<td>0.036/0.028</td>
<td>No (7/10)</td>
<td>Yes (3/8)</td>
</tr>
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<td>97157413</td>
<td>rs121990352</td>
<td>G/T</td>
<td>Missense</td>
<td>0.016/0.003³</td>
<td>No (4/6)³</td>
<td>Unknown (0/3)</td>
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<td>T/A</td>
<td>Intronic</td>
<td>0.005/NA</td>
<td>No (1/2)</td>
<td>Yes (1/2)</td>
</tr>
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<td>g.106+2780C&gt;T</td>
<td>C/T</td>
<td>Intronic</td>
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<td>Yes (2/2)</td>
<td>Yes (1/2)</td>
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<tr>
<td>GDF6</td>
<td>8</td>
<td>97170374</td>
<td>rs140757891</td>
<td>C/T</td>
<td>Intronic</td>
<td>0.021/0.013</td>
<td>Yes (4/4)</td>
<td>Yes (2/10)</td>
</tr>
<tr>
<td>GDF3</td>
<td>12</td>
<td>7842587</td>
<td>rs2302516</td>
<td>C/G</td>
<td>Missense</td>
<td>0.047/0.024</td>
<td>No (7/11)</td>
<td>Yes (1/14)</td>
</tr>
</tbody>
</table>

⁹Only variants which were followed-up are shown here (See Methods section); Variants were validated by bidirectional sequencing and all sampled affected and
unaffected individuals within each identified family were sequenced.
¹Base pair positions based on human genome build GRCh37/hg19.
The nomenclature used to describe novel variants was based on recommendations by the Human Genome Variation Society (den Dunnen and Antonarakis 2001).
Nucleotide numbering was based on the GDF6 RefSeq genomic sequence, NG_008981.1, and intron-exon boundaries were defined based on the GDF6 mRNA
sequence, NM_001001557.
²Alleles: Reference allele/Alternate allele.
³CMI MAF estimate based on all affected family members initially screened; 1KG MAF: Based on 1000 Genomes Integrated Phase 1 Release v3: European population.
⁴Is sharing observed across all affected individuals within each family?
⁵Numbers in parentheses: Numerator: number of sampled individuals carrying the variant, Denominator: total number of sampled individuals. Only affecteds were
considered for “All affected” and only unaffecteds/uncertains were considered for “Reduced penetrance”.
⁶MAF estimate was not available from 1000 Genomes; MAF estimate based on the Exome sequencing project: European population.
⁷Individual suspected to have Chiari Malformation Type 0 is counted as “affected” for the purposes of this table.

doi:10.1371/journal.pone.0061521.t003
detection. Other candidate genes, such as low density lipoprotein receptor-related protein 6 (LRP6) present within the chromosome 12 candidate interval could also be investigated as LRP6, when specifically deleted from early mesenchyme, causes a slight delay in mouse skull ossification [53]. In addition, rather than simply taking a candidate gene approach, targeted capture and next generation sequencing of candidate genomic intervals defined by linkage analysis or whole genome sequencing would be an obvious next step to comprehensively follow-up these findings. Finally, taking a more quantitative approach to disease, for example by focusing on cranial base morphometrics, may yield greater insight into the genetic etiology due to increased statistical power and reduced misclassification rates among individuals.

Conclusion

The current study demonstrates the utility of using clinical stratification to reduce genetic heterogeneity in CMI by identifying genomic regions showing increased evidence for linkage with maximum LOD scores exceeding 2 and even 3, as well as having implicated credible candidate genes in CMI susceptibility. Although further work is necessary to confirm the involvement of these genes and individual sequence variants in the development of CMI, this work makes several important contributions to the field of CMI research: 1) We conducted the largest whole genome linkage screen to date providing multiple candidate intervals for future investigation and replication, 2) Our results suggest a relationship between CTD related conditions and genetic etiology which is consistent with the hypothesis that CMI with CTDs versus CMI without CTDs occurs through different mechanisms ("cranial settling" versus "cranial constriction"), 3) Multiple biological candidates were implicated from the analysis, including the only two GDFs currently known to be associated with KFS suggesting a shared genetic etiology between CMI and KFS. This is consistent with the fact that KFS is known to co-occur with CMI and share a common cellular etiology, 4) Identified a known KFS missense mutation in two of our families that is not necessary for disease but likely contributes to the phenotype due to its rare frequency in the general population, known functional effect in vitro, and the fact that it has been identified in multiple skeletal and ocular disease cohorts, and 5) Identified two potential regulatory variants (one novel, one rare) shared across all affected individuals in the families they were identified in and located within predicted regulatory regions for SUZ12 which itself is an excellent candidate gene for CMI. Further investigation of GDF3 and GDF6, other plausible biological candidates such as SUZ12, NF1, and LRP6, as well as the genetic relationship between CMI and KFS is warranted.

Supporting Information

Figure S1 Multidimensional scaling (MDS) analysis. This was performed using a pruned marker dataset and only one representative individual from each family. The red and black colors correspond to different clusters (PLINK’s pairwise population concordance test: –ppc 1e-4). Individuals shown in red represent families that are self-reported Caucasian, Hispanic. MDS plot was created in R 2.15.0.

Figure S2 Segregation of select variants in three CMI pedigrees. Family 9772 (A), Family 9496 (B), and Family 9432 (C). Alleles “A” and “a” represent a novel SNP at Chr8:97154395, Alleles “B” and “b” represent a novel SNP at Chr8:97157811, alleles “C” and “c” represent RS140757891, and alleles “D” and “d” represent a novel SNP at Chr8:97169735. Individual 108 from family 9496 has previously had brain surgery and a shunt; no additional information is known. Symbols shaded in black indicate a diagnosis of CMI with or without syringomyelia and small diamonds represent miscarriages. Lower case letters shown in red indicate the variant allele. Genotype calls are based on bi-directional sequencing. Progeny 8 (Delray Beach, FL) was used to construct the pedigrees.

Table S1 PCR primer sequences and conditions.

Table S2 PCR primer kits and thermocycler conditions.

Table S3 Quality control of sample data.

Table S4 Most significant two-point and multipoint LOD scores.

Table S5 GDF6 and GDF3 identified sequence variants.

Acknowledgments

We would like to thank all family members for participating in the Chiari genetics study. In addition, we acknowledge the technical assistance received from Carol Haynes, Colette Blach, and Blair Chesnut at the Duke Center for Human Genetics.

Author Contributions

Conceived and designed the experiments: CAM SGG AAK. Performed the experiments: CAM KS KD EA JS. Analyzed the data: CAM. Contributed reagents/materials/analysis tools: DE GG HF SGG AAK. Wrote the paper: CAM KS KD HC EA JS DE GG HF SGG AAK.

References


Genetic Evaluation and Application of Posterior Cranial Fossa Traits as Endophenotypes for Chiari Type I Malformation

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Summary

Chiari Type I Malformation (CMI) is characterized by herniation of the cerebellar tonsils through the base of the skull. Although cerebellar tonsillar herniation (CTH) is hypothesized to result from an underdeveloped posterior cranial fossa (PF), patients are frequently diagnosed by the extent of CTH without cranial morphometric assessment. We recently completed the largest CMI whole genome qualitative linkage screen to date. Despite an initial lack of statistical evidence, stratified analyses using clinical criteria to reduce heterogeneity resulted in a striking increase in evidence for linkage. The present study focused on the use of cranial base morphometrics to further dissect this heterogeneity and increase power to identify disease genes. We characterized the genetic contribution for a series of PF traits and evaluated the use of heritable, disease-relevant PF traits in ordered subset analysis (OSA). Consistent with a genetic hypothesis for CMI, much of the PF morphology was found to be heritable and multiple genomic regions were strongly implicated from OSA, including regions on Chromosomes 1 (LOD = 3.07, p = 3 × 10⁻³) and 22 (LOD = 3.45, p = 6 × 10⁻⁵) containing several candidates warranting further investigation. This study underscores the genetic heterogeneity of CMI and the utility of PF traits in CMI genetic studies.

Keywords: Posterior cranial fossa, Chiari Type I Malformation, endophenotypes, heritability, ordered subset analysis

Introduction

Chiari Type I Malformation (CMI) is characterized by the displacement of the cerebellar tonsils below the base of the skull. The disease prevalence is estimated to be slightly less than 1% in the United States (Meadows et al., 2000; Speer et al., 2003), although this is likely an underestimate as the gold standard for diagnosis requires the use of magnetic resonance imaging (MRI) to determine the extent to which the cerebellar tonsils are herniated and asymptomatic individuals are unlikely to undergo MRI. Patients with CMI can present with a wide range of neurological symptoms and conditions, some of which can cause significant morbidity. Currently, the only form of treatment to alleviate symptoms is to perform a posterior fossa (PF) decompression surgery. The goal of this surgery is to expand the cranial base to provide additional room for the cerebellum while also restoring normal cerebrospinal fluid flow.

Although several lines of evidence exist supporting a genetic contribution to disease, including familial aggregation, twin studies, and co-occurrence with known genetic syndromes [for review see (Markunas et al., 2013)], limited research has been conducted investigating the genetic basis of this debilitating disorder. In 2006, Boyles and colleagues performed a whole genome linkage screen using 23 non-syndromic, CMI multiplex families, and identified significant evidence for linkage to regions on Chromosomes 9 and 15 (Boyles et al., 2006). In addition, a heritability analysis...
was conducted using 11 PF measurements. PF volume (PFV) and basal angle were significantly heritable within the families ($p < 0.05$), while the clivus and supraoccipital bone lengths trended toward being significant ($p < 0.10$). These were particularly important findings as the primary mechanism hypothesized for “classical” CMI is “cranial constriction” (Milhorat et al., 2010). Under this mechanism, underdeveloped occipital bones are thought to lead to a compromised PF (cranial constriction) resulting in tonsillar herniation occurring secondarily (Marin-Padilla & Marin-Padilla, 1981; Nishikawa et al., 1997). In addition to this whole genome linkage screen and heritability study, a case-control genetic association study examining 58 candidate genes was recently conducted (Urbizu et al., 2013). Although no associations met adjustment for multiple testing within the entire study population, when analyses were restricted to 186 patients with a small posterior fossa they found four SNPs in three genes ($CDX1$, $FLT1$, $ALDH1A2$) that remained significant (FDR 10%). Aside from the family study conducted by Boyles and colleagues, the only other whole genome screen conducted to date was recently published by our group (Markunas et al., 2013). Using 66 nonsyndromic, CMI multiplex families we conducted a whole genome qualitative linkage screen followed by a stratified analysis based on clinical criteria in order to reduce phenotypic and potentially genetic heterogeneity. This resulted in increased evidence for linkage to several regions of the genome within family subsets that were clinically more similar. Of particular interest were two regions on Chromosomes 8 and 12 that harbored the growth differentiation factor genes, $GDF6$ and $GDF3$, previously implicated in Klippel–Feil syndrome which is diagnosed in roughly 3–5% of CMI patients (Milhorat et al., 1999; Tubbs et al., 2011). In order to investigate the possibility that these are allelic disorders, $GDF3$ and $GDF6$ were sequenced and several $GDF6$ variants of potential significance were identified.

As described earlier, CMI is a phenotypically heterogeneous disorder with patients exhibiting high degrees of variation with respect to symptom presentation, age of disease onset, presence of associated conditions, and response to surgery. This phenotypic heterogeneity may be related to etiologic variability, or the presence of multiple environmental and genetic factors contributing to disease. Endophenotypes, or quantitative intermediate phenotypes have been widely applied in the psychiatric literature (Gottesman & Gould, 2003) and can be a powerful tool to help address phenotypic heterogeneity by being more directly related to the underlying disease mechanism and etiologic factors as compared to the qualitative disease outcome. Candidate endophenotypes should be heritable, quantitative, and associated with the disease. Cranial base morphological traits, such as PF measurements, represent excellent candidate endophenotypes for CMI. PF traits are relevant to the primary disease mechanism and are therefore more likely to be strongly associated with underlying genetic factors than the qualitative disease outcome, which is complicated by phenotypic heterogeneity and a lack of consistent diagnostic criteria. Furthermore, PF measurements have been shown to vary across CMI patients grouped according to the presumed cerebellar tonsillar herniation (CTH) mechanism (Milhorat et al., 2010), suggesting that use of appropriate PF traits in stratified analyses may identify mechanistically and perhaps etiologically similar families, ultimately resulting in increased power to identify disease genes.

In the present study, a comprehensive genetic evaluation of a series of PF traits was first carried out to determine which components of the PF were heritable. Candidate endophenotypes, as defined by those PF traits that were both heritable and associated with CMI, were then selected for use in ordered subset analysis (OSA; Hauser et al., 2004) to further refine our previously conducted whole genome linkage screen and increase our power to identify disease genes (Markunas et al., 2013). The approach used in this study was motivated by several key factors: (1) CMI is a heterogeneous disorder, as is evident by the extent of phenotypic variability observed across patients (phenotypic heterogeneity) and the initial lack of statistical evidence from our previous whole genome linkage screen when the complete collection of families was used (likely due to genetic heterogeneity), (2) Using the same set of families, stratified linkage analyses using clinical criteria related to proposed mechanistic differences between patients led to a significant increase in evidence for linkage to multiple genomic regions and the successful identification of several convincing biological candidates, and (3) PF plays a role in CMI susceptibility and previous work suggests that specific PF traits may be used to differentiate CMI patients with different underlying disease mechanisms. Importantly, we found that the use of heritable, disease-relevant PF traits in OSA led to the identification of several genomic regions showing increased evidence for linkage as well as several promising candidate genes which will be discussed in detail along with their potential association with the PF trait of interest.

**Materials and Methods**

**Patient Population**

Study population characteristics and ascertainment for the study have previously been described in detail (Markunas et al., 2013). Briefly, families were enrolled if at least two individuals presented with CMI with or without syringomyelia. Families with a positive history of a genetic syndrome previously associated with CMI or individuals thought to have secondary forms of CMI were excluded. The average number of affected individuals per family was 2.77 ± 0.99 with a range spanning from 2 to 6. All available presurgical brain
MRIs were collected on affected and unaffected family members. In total, 120 MRIs were obtained from 92 affected and 28 unaffected individuals from 50 of the 66 families (mean age at MRI = 29.23 ± 19.05 years; 83 females and 37 males). A diagnosis of CMI was made based on MRI measurements in which affection status was defined as CTH of 3 mm or more for both tonsils (Barkovich et al., 1986) or herniation of 5 mm or more for either tonsil (Aboulezz et al., 1985). If MRIs were unavailable, a diagnosis was based on medical records, followed by patient report. All participating family members provided written informed consent that had been approved by the Institutional Review Board of Duke University Medical Center.

**Posterior Cranial Fossa Measurements**

Using a team-based approach, two trained researchers took a series of PF measurements from the sagittal midline of a T1-weighted MRI, unless described otherwise (Fig. 1). All measurements were verified by a board certified neuroradiologist blinded to the clinical data (D.E.). Herniation of the left and right tonsils was measured by a line drawn from the tips of the cerebellar tonsils perpendicularly to the foramen magnum on a sagittal image to the left and right of the midline, respectively. Four cranial base angle measurements were made: (1) Basal angle: angle between a line extending from the basion to the center of the sella turcica and a line extending from the sella turcica to the nasion, (2) Boogaard’s angle: angle between the clivus and the foramen magnum, (3) Occipital angle: angle between the supraoccipital bone and the foramen magnum, and (4) Tentorial angle: angle between the supraoccipital bone and the tentorium. The length of the clivus was defined by a line drawn from the basion to the top of the dorum sella. A line created from the basion to the opisthion determined the anterior posterior distance of the foramen magnum. The supraoccipital bone was measured from the opisthion to the center of the internal occipital protuberance. The tentorium was defined by a line extending out from the center of the internal occipital protuberance to just posterior of the vein of Galen. The line connecting the tip of the tentorium to the top of the dorum sella was described as the “tentorial opening.” PF height was determined by a line extending from the tip of the tentorium perpendicularly to the level of the foramen magnum.

The tentorial opening, tentorium, supraoccipital bone, foramen magnum, and clivus were used as borders to estimate PF area (PFA). In order to evaluate the PFA, a reference line was first created by drawing a line from the top of the dorum sella to the center of the internal occipital protuberance. Subsequently, four additional measurements were made: (1) line drawn perpendicularly from the basion to the reference line (BasToRef), (2) line drawn perpendicularly from the opisthion to the reference line (OpisToRef), (3) line drawn perpendicularly from the tip of the tentorium to the reference line (TentToRef), and (4) distance between BasToRef and OpisToRef along the reference line (Trapheight) (Fig. 1).
The area corresponding to the top of the PF (above the reference line) was estimated by the sum of areas 4 and 5, the area representing the bottom of the PF (below the reference line) was estimated by the sum of areas 1, 2, and 3, and the total PFA was estimated as the sum of areas 1 to 5 (Fig. 1).

PFV was estimated from serial axial T1-weighted MRI slices taken from the foramen magnum to the top of the PF. In order to obtain an estimate of the PFV, the area of the PF was estimated for each axial image and then multiplied by the slice thickness. To account for gaps between slices, when present, the mean area of the adjacent slices was calculated and then multiplied by the distance between the slices. Total PFV was estimated by summing over all volumes. This was performed on a subset of individuals (N = 19) in order to estimate the correlation between PFV and the other PF measurements described earlier. Summary statistics and a Pearson correlation matrix for the PF traits were calculated using SAS 9.3 (Cary, NC, USA).

Principal Components Analysis

Since a large number of PF traits are moderately to highly correlated with one another, principal components analysis (PCA) was performed in order to create a smaller number of uncorrelated, composite traits as has been done previously, for example, with skeletal traits (Chase et al., 2002) and metabolomic data (Shah et al., 2009). Prior to performing PCA, outliers as defined by > 4 standard deviations away from the mean were first excluded. Studentized residuals were then calculated for each PF trait from linear regression models adjusted for age at MRI, measurer, and sex using SAS 9.3 (SAS, Cary, NC, USA). For PCA, most PF traits were included (Nincluded = 15/26) with the following exceptions: only one of the CTH measurements (maximum herniation) and none of the area estimates were incorporated. PCA was performed using proc factor (method = p) in SAS 9.3 with the studentized residuals as input. The top PCs were retained for further analysis based on the Kaiser criterion (eigenvalue > 1). Varimax rotation was performed in order to aid in the interpretability of the PCs. PF traits with a rotated factor load ≥ 0.4 were assigned to each PC as the primary contributors. Component scores were computed and kept in order to estimate heritability as described later.

Heritability Estimates

Heritability, which refers to the proportion of phenotypic variation that is attributable to genetic variation, was estimated for all PF measurements as well as the PCs with an eigenvalue > 1 using SOLAR 4.2.0 (Almasy & Blangero, 1998). As was performed prior to PCA, outliers for each individual PF measurement were identified as defined by > 4 standard deviations outside of the mean and removed prior to the heritability analysis. This resulted in only three PF measurements, each from different individuals, being excluded. Once outliers were removed if the raw trait values resulted in a residual kurtosis within the normal range, they were left untransformed for the heritability analysis (56.25% PF traits).

For several of the remaining traits, a natural log transformation alone was not sufficient in order to achieve a residual kurtosis within the normal range, thus we performed one of the following: (1) removed 1–4 extreme values, or (2) removed 1 extreme value followed by a natural log transformation if the first approach was unsuccessful. Forty four percent of the PF traits needed one (9.38%), two (9.38%), three (3.13%), or four (12.50%) extreme values removed. After the removal of 1 extreme value for left herniation, maximum herniation, and minimum herniation, a constant (10) was added prior to performing a natural log transformation. Natural-log transformed values were then multiplied by a factor of 12 in order to achieve a standard deviation > 0.5.

For the heritability analysis, the polygenic command in SOLAR was used which provides an estimate of the total additive genetic heritability (Almasy & Blangero, 1998). For individual PF traits, the heritability analysis included age at MRI, sex, affection status, and measurer as covariates. For the PCs, the heritability analysis only adjusted for affection status since variation due to age at MRI, sex, and measurer had previously been removed as described above. In order to reduce the potential for ascertainment bias, ascertainment was corrected for by conditioning on the proband for all analyses (Almasy & Blangero, 1998).

A bivariate polygenic analysis was also conducted in SOLAR 4.2.0 for select traits. SOLAR uses a maximum likelihood variance decomposition method in order to estimate genetic and environmental correlations between two quantitative traits (Almasy et al., 1997). This approach was used to estimate the genetic correlation between candidate traits selected for use in OSA described later.

Ordered Subset Analysis

We previously conducted a whole genome linkage screen using 367 individuals from 66 families presenting with nonsyndromic CMI with or without syringomyelia (Markunas et al., 2013). Detailed study design, quality control procedures, and analytical methods have been described previously (Markunas et al., 2013). Briefly, family members were genotyped using the IlluminaHuman610-Quad BeadChips (Illumina Inc., San Diego, CA, USA). Two-point and multipoint parametric and nonparametric linkage analyses were conducted using Merlin 1.1.2 and MINX (MERLIN in X) (Abecasis et al., 2002).
Table 1  Summary of MRI measurements.

<table>
<thead>
<tr>
<th>MRI Measurement</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right herniation (mm)</td>
<td>118</td>
<td>0</td>
<td>26.5</td>
<td>7.30</td>
<td>6.09</td>
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<tr>
<td>Left herniation (mm)</td>
<td>119</td>
<td>0</td>
<td>28.4</td>
<td>7.20</td>
<td>6.12</td>
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<tr>
<td>Minimum herniation (mm)</td>
<td>120</td>
<td>0</td>
<td>26.5</td>
<td>6.45</td>
<td>5.79</td>
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<tr>
<td>Maximum herniation (mm)</td>
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<td>0</td>
<td>28.4</td>
<td>7.94</td>
<td>6.28</td>
</tr>
<tr>
<td>Foramen magnum (mm)</td>
<td>120</td>
<td>27.9</td>
<td>46</td>
<td>37.79</td>
<td>3.02</td>
</tr>
<tr>
<td>Tentorium (mm)</td>
<td>120</td>
<td>38.86</td>
<td>62.4</td>
<td>48.61</td>
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<tr>
<td>Supraoccipital bone (mm)</td>
<td>120</td>
<td>20</td>
<td>51</td>
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<td>Clivus (mm)</td>
<td>120</td>
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<td>53</td>
<td>40.94</td>
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<td>119</td>
<td>37.7</td>
<td>68</td>
<td>55.22</td>
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<td>120</td>
<td>40</td>
<td>80.9</td>
<td>61.06</td>
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<td>16</td>
<td>40</td>
<td>30.70</td>
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<td>19</td>
<td>45</td>
<td>29.69</td>
<td>4.40</td>
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<td>Trapezoid height (mm)</td>
<td>120</td>
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<td>46.7</td>
<td>37.48</td>
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<td>Tentorial angle (°)</td>
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<td>73</td>
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<td>Occipital angle (°)</td>
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<td>129.33</td>
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<td>Basal angle (°)</td>
<td>116</td>
<td>110</td>
<td>145</td>
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<td>119</td>
<td>106</td>
<td>148.59</td>
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<td>8.51</td>
</tr>
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<td>144.62</td>
<td>702.25</td>
<td>398.33</td>
<td>92.45</td>
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<td>1656</td>
<td>1193.44</td>
<td>182.07</td>
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<td>96</td>
<td>650.25</td>
<td>353.01</td>
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<td>Area4 (mm)</td>
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<td>1147.06</td>
<td>685.14</td>
<td>133.76</td>
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<td>Area5 (mm)</td>
<td>120</td>
<td>345.15</td>
<td>918.7</td>
<td>568.72</td>
<td>112.93</td>
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<td>Posterior fossa area (PFA) (cm)</td>
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<td>146.46</td>
<td>390.05</td>
<td>320.00</td>
<td>35.49</td>
</tr>
<tr>
<td>PFA above reference line (mm)</td>
<td>120</td>
<td>553.28</td>
<td>1934.24</td>
<td>1253.86</td>
<td>208.64</td>
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<td>PFA below reference line (mm)</td>
<td>120</td>
<td>911.35</td>
<td>2678.44</td>
<td>1944.78</td>
<td>272.66</td>
</tr>
</tbody>
</table>

Abbreviations: MRI, magnetic resonance images; Min, minimum; Max, Maximum; Std dev, standard deviation; mm, millimeter; cm, centimeter.

For the nonparametric analysis (NPL), the $S_{all}$ scoring function was used to assess IBD sharing across subsets of individuals (Whittmore & Halpern, 1994). In order to evaluate statistical significance, both the Kong and Cox linear and exponential models were applied (Kong & Cox, 1997), although only the linear model will be used in the present study.

As genetic heterogeneity can negatively impact a linkage screen, we sought to reduce potential heterogeneity across our collection of families by performing OSA (Hauser et al., 2004) using PF traits that were found to be both significantly heritable and associated with affection status (nominal $p$-value < 0.05). As previously described by Hauser et al. (2004), families were ordered using a trait-related covariate (e.g., PF traits) to identify a subset of families providing maximal evidence for linkage. A permutation test was then performed to assess the significance of the increased evidence for linkage provided by the subset of families (Hauser et al., 2004).

For individual PF traits, a single covariate value for each family was calculated as follows: (1) Studentized residuals were calculated for each PF trait from linear regression models adjusted for age at MRI, measurer, and sex using SAS 9.3, and (2) A family-level value was calculated by taking the median across affected family members; a single value was used if only one affected individual was available. For the composite PF traits (PCs), family-level values were calculated by taking the median of the component scores across affected family members; a single value was used if only one affected individual was available.

OSA was performed using multipoint NPL partial LOD (pLOD) scores obtained under a linear model and PF trait values for each family were used to rank families in both ascending and descending order.

Results

Posterior Fossa Traits

Descriptive statistics of the individual PF traits after outlier removal can be found in Table 1. A Pearson correlation matrix of PF traits is also provided as supplementary material (Table S1). As PF volume is relatively labor intensive to generate and difficult to assess, we examined the correlation between PF
volume and the remaining PF traits using a smaller number of individuals to determine if any of those traits could act as a reasonable proxy for PF volume. The top five most correlated PF traits included PFA ($\rho = 0.70, p = 0.0008$), PFA below the reference line ($\rho = 0.61, p = 0.006$), area2 ($\rho = 0.54, p = 0.018$), PF height ($\rho = 0.46, p = 0.046$), and area1 ($\rho = 0.46, p = 0.048$). Thus, given the high degree of correlation between PF area and PF volume and the fact that, unlike PF volume, PF area is not as challenging to examine, the present study does not include PF volume in subsequent analyses.

Principal Components Analysis

PCA was performed using studentized residuals obtained for each of the 15 PF traits from linear regression models as described in Materials and Methods. The top six PCs with an eigenvalue > 1 were retained for further analysis. Collectively these PCs account for 86.8% of the total variation in the traits. In order to aid in the interpretation of the PCs, a visual representation of each of the top six PCs is provided highlighting the top weighted PF traits (Fig. 2).

Heritability Estimates

A summary of the results from the heritability analysis is shown in Table 2. In order to account for multiple testing, a bonferroni correction was applied to the data (Table 2). However, as many of the traits are moderately to highly correlated with one another (Table S1), this is an extremely conservative approach thus findings should be interpreted accordingly. When considering 29 PF traits (maximum herniation was selected to represent the four tonsillar herniation measurements), 72.4% of the traits were found to be heritable (nominal $p < 0.05$). Of those found to be heritable, twelve traits were significantly associated with affection status, including PF height, PC4, area5, basal angle, supraoccipital bone, opisthion to reference, PC1, PF area, basion to reference, area2, PC6, and PC3.

Ordered Subset Analysis

A summary of the significant (nominal empirical $p$-value $< 0.05$) OSA results are provided in Table 3, along with information regarding the number of families in the OSA subset and the PF trait used to identify them. Importantly, the families present in the OSA subset that provide maximal evidence for linkage are not necessarily the largest, or most powerful but instead are more genetically homogeneous resulting in increased power even though the overall sample size has been decreased. Only one result on Chromosome 22 (Table 3, Fig. 3) withstood a bonferroni correction accounting for 552 tests (23 chromosomes $\times$ 12 PF traits $\times$ 2 OSA covariate orders, ascending and descending). However, as stated previously, many of these traits are highly correlated with one another (Table S1) thus a bonferroni correction is extremely conservative and should be taken into consideration when interpreting these findings. The top three most significant OSA findings were for PF height, area5, and PC4, all of which showed increased evidence for linkage (Max LOD $> 3$, emp $p$-value $< 0.0008$) to an overlapping region on Chromosome 22 (cM $= 50.2–52$) within a subset of families characterized...
### Table 2 Heritability analysis of posterior fossa traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>H2</th>
<th>H2 SE</th>
<th>H2 PVAL</th>
<th>Age PVAL</th>
<th>Sex PVAL</th>
<th>CMI PVAL</th>
<th>IND PVAL</th>
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<tbody>
<tr>
<td>PF height</td>
<td>0.77</td>
<td>0.19</td>
<td>1.74E-04</td>
<td>0.33</td>
<td>0.92</td>
<td>1.63E-05</td>
<td>0.56</td>
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<tr>
<td>PC4</td>
<td>0.72</td>
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<td>1.24E-03</td>
<td>N/A</td>
<td>N/A</td>
<td>9.44E-03</td>
<td>N/A</td>
</tr>
<tr>
<td>Tentorium to reference</td>
<td>0.67</td>
<td>0.24</td>
<td>1.52E-03</td>
<td>0.79</td>
<td>0.54</td>
<td>0.15</td>
<td>0.35</td>
</tr>
<tr>
<td>Area5</td>
<td>0.68</td>
<td>0.24</td>
<td>1.70E-03</td>
<td>0.96</td>
<td>0.02</td>
<td>0.046</td>
<td>0.24</td>
</tr>
<tr>
<td>Basal angle</td>
<td>0.61</td>
<td>0.22</td>
<td>2.52E-03</td>
<td>0.35</td>
<td>0.91</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>PFA above reference</td>
<td>0.70</td>
<td>0.28</td>
<td>3.71E-03</td>
<td>0.41</td>
<td>0.13</td>
<td>0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>PC2</td>
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<td>4.40E-03</td>
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<td>N/A</td>
<td>0.958</td>
<td>N/A</td>
</tr>
<tr>
<td>Tentorium</td>
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<td>6.22E-03</td>
<td>0.75</td>
<td>6.82E-03</td>
<td>0.09</td>
<td>0.30</td>
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<tr>
<td>Supraoccipital bone</td>
<td>0.64</td>
<td>0.26</td>
<td>8.59E-03</td>
<td>0.62</td>
<td>0.11</td>
<td>0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>Opiolith to reference</td>
<td>0.53</td>
<td>0.22</td>
<td>0.01</td>
<td>0.61</td>
<td>0.35</td>
<td>2.58E-03</td>
<td>0.20</td>
</tr>
<tr>
<td>Boogaard’s angle</td>
<td>0.47</td>
<td>0.22</td>
<td>0.01</td>
<td>0.92</td>
<td>0.92</td>
<td>0.13</td>
<td>0.35</td>
</tr>
<tr>
<td>Area3</td>
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<td>0.26</td>
<td>0.02</td>
<td>0.86</td>
<td>0.09</td>
<td>0.12</td>
<td>0.62</td>
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<tr>
<td>Tentorial opening</td>
<td>0.46</td>
<td>0.23</td>
<td>0.02</td>
<td>0.14</td>
<td>0.80</td>
<td>0.84</td>
<td>0.55</td>
</tr>
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<td>PC1</td>
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<td>0.23</td>
<td>0.018</td>
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<td>N/A</td>
<td>0.019</td>
<td>N/A</td>
</tr>
<tr>
<td>PFA</td>
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<td>0.23</td>
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<td>0.25</td>
<td>0.96</td>
<td>2.33E-04</td>
<td>0.54</td>
</tr>
<tr>
<td>Area2</td>
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<td>0.23</td>
<td>0.03</td>
<td>0.17</td>
<td>0.06</td>
<td>4.47E-03</td>
<td>0.97</td>
</tr>
<tr>
<td>Trapezoid height</td>
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<td>0.18</td>
<td>0.03</td>
<td>0.22</td>
<td>3.15E-05</td>
<td>0.15</td>
<td>0.85</td>
</tr>
<tr>
<td>PC5</td>
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<td>N/A</td>
<td>N/A</td>
<td>0.721</td>
<td>N/A</td>
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<tr>
<td>PC6</td>
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<td>0.17</td>
<td>0.043</td>
<td>N/A</td>
<td>N/A</td>
<td>1.9E-08</td>
<td>N/A</td>
</tr>
<tr>
<td>PC3</td>
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<td>0.28</td>
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<td>N/A</td>
<td>0.019</td>
<td>N/A</td>
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<tr>
<td>Foramen magnum</td>
<td>0.29</td>
<td>0.18</td>
<td>0.05</td>
<td>0.29</td>
<td>1.90E-07</td>
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<tr>
<td>Area4</td>
<td>0.43</td>
<td>0.30</td>
<td>0.06</td>
<td>0.33</td>
<td>0.59</td>
<td>0.66</td>
<td>0.42</td>
</tr>
<tr>
<td>PFA below reference line</td>
<td>0.30</td>
<td>0.25</td>
<td>0.11</td>
<td>0.04</td>
<td>0.22</td>
<td>1.17E-03</td>
<td>0.87</td>
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<tr>
<td>Tentorial angle</td>
<td>0.18</td>
<td>0.24</td>
<td>0.23</td>
<td>0.84</td>
<td>0.35</td>
<td>0.05</td>
<td>0.75</td>
</tr>
<tr>
<td>Occipital angle</td>
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<td>0.23</td>
<td>0.37</td>
<td>0.57</td>
<td>0.97</td>
<td>0.73</td>
<td>2.97E-03</td>
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<tr>
<td>Clivus</td>
<td>0.04</td>
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<td>0.43</td>
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<td>2.45E-03</td>
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<tr>
<td>Minimum herniation</td>
<td>0</td>
<td>N/A</td>
<td>0.5</td>
<td>0.13</td>
<td>0.15</td>
<td>4.04E-19</td>
<td>0.11</td>
</tr>
<tr>
<td>Left herniation</td>
<td>0</td>
<td>N/A</td>
<td>0.5</td>
<td>0.26</td>
<td>0.11</td>
<td>7.38E-18</td>
<td>0.42</td>
</tr>
<tr>
<td>Maximum herniation</td>
<td>0</td>
<td>N/A</td>
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<td>0.21</td>
<td>0.18</td>
<td>7.58E-18</td>
<td>0.13</td>
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<tr>
<td>Right herniation</td>
<td>0</td>
<td>N/A</td>
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<td>0.07</td>
<td>0.13</td>
<td>6.38E-13</td>
<td>0.03</td>
</tr>
<tr>
<td>Area1</td>
<td>0</td>
<td>N/A</td>
<td>0.5</td>
<td>8.01E-04</td>
<td>0.15</td>
<td>5.13E-03</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Table shown in descending order by H2 PVAL.
Nominal p-values < 0.05 are shown in bold. Tests meeting a conservative Bonferroni correction for 32 comparisons are underlined (p < 0.0016).

Abbreviations: PF, posterior fossa; PFA, PF area; PC, principal component; H2, heritability estimate; H2 SE, standard error on heritability estimate; PVAL, p-value; CMI, Chiari Malformation Type I; N/A, not applicable; ref, reference; IND, measurer.

by large values for PF height, area5, and PC4. Interestingly, these three PF traits were also among the most significantly heritable traits in our families (h² > 0.65, p < 0.002). In order to further assess the relationship among these traits, the genetic correlation between the most significant trait, PF height, and the remaining 11 PF traits selected for OSA was computed. Only three PF traits had a genetic correlation (ρg) exceeding 0.75 with PF height (ρg < 0.40 for all other traits): (1) PF area (ρg = 0.85, standard error (SE) = 0.14), (2) PC4 (ρg = 0.80, SE = 0.14), and (3) area5 (ρg = 0.78, SE = 0.16). Although the use of PF area as a covariate for OSA did not lead to one of the most significant findings overall, when families were arranged in descending order by PF area increased evidence for linkage to the same general region on Chromosome 22 was found (Max LOD = 2.10, emp p-value = 0.025, position = 49.9 cM). The candidate interval identified on Chromosome 22 (defined by LOD > 2) by PF height spans 10.2 Mb (GRCh37 genomic coordinates: 36590946–46769741) and contains 205 known RefSeq genes (based on the UCSC genome browser; 09/26/2012).

An additional finding of interest was found on Chromosome 1 (Table 3, Fig. 3) within a subset of families defined by small basal angle values (Max LOD = 3.07, emp p-value = 0.0032, position = 182.8 cM). Out of the...
Table 3 Summary of OSA results.

<table>
<thead>
<tr>
<th>CHR</th>
<th>POS</th>
<th>Covariate</th>
<th>Order</th>
<th>UNCOND LOD</th>
<th>MAX LOD</th>
<th>PERMS</th>
<th>EMP PVAL</th>
<th>N SUBSET FAMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>50.2</td>
<td>PFH</td>
<td>H to L</td>
<td>0.13</td>
<td>3.45</td>
<td>10000</td>
<td>6.0E-05</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>52.0</td>
<td>AREA5</td>
<td>H to L</td>
<td>0.11</td>
<td>3.17</td>
<td>10000</td>
<td>2.0E-04</td>
<td>12</td>
</tr>
<tr>
<td>22</td>
<td>52.0</td>
<td>PC4</td>
<td>H to L</td>
<td>0.21</td>
<td>3.17</td>
<td>10000</td>
<td>7.0E-04</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>182.8</td>
<td>BASAL_ANG</td>
<td>L to H</td>
<td>0.32</td>
<td>3.07</td>
<td>5560</td>
<td>3.2E-03</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>126.7</td>
<td>OPISTOREF</td>
<td>L to H</td>
<td>0.35</td>
<td>2.78</td>
<td>4620</td>
<td>3.9E-03</td>
<td>21</td>
</tr>
<tr>
<td>13</td>
<td>128.4</td>
<td>PC3</td>
<td>L to H</td>
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<tr>
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<td>L to H</td>
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<td>1090</td>
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<td>19</td>
</tr>
<tr>
<td>10</td>
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<td>AREA5</td>
<td>L to H</td>
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<tr>
<td>8</td>
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<tr>
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<td>1.70</td>
<td>1000</td>
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<tr>
<td>10</td>
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<td>PC4</td>
<td>L to H</td>
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<td>2.29</td>
<td>1000</td>
<td>1.0E-04</td>
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<td>H to L</td>
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<td>2.70</td>
<td>1000</td>
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<tr>
<td>10</td>
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<td>L to H</td>
<td>0.22</td>
<td>2.35</td>
<td>1000</td>
<td>1.0E-05</td>
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<td>2.65</td>
<td>1000</td>
<td>1.0E-05</td>
<td>21</td>
</tr>
</tbody>
</table>

Only results with a nominal empirical \(p\)-value less than 0.05 are shown.
Empirical \(p\)-values that meet a bonferroni correction for 552 tests are bold and underlined; empirical \(p\)-values < 0.05 are bold.
LOD scores \(\geq 3\) are bold and underlined; LOD scores \(\geq 2\) are bold.

1Position (POS) refers to the maximum LOD score location in centimorgans.

2Covariate refers to the PF trait used to order families for OSA.

3Order: H to L: families were ordered by the covariate in descending order; L to H: families were ordered in ascending order.

4The total number of families used in the analysis varied by PF trait. In general, the total number of available families was 49 and 47 for non-PC and PC traits, respectively.

Abbreviations: CHR, chromosome; UNCOND LOD, unconditional LOD score (obtained using all families); MAX LOD, maximum LOD score (obtained using a subset of families); PERMS, number of permutations; EMP PVAL, empirical \(p\)-value; N SUBSET FAMS, number of families selected in the OSA subset; PF, posterior fossa.

PF traits used in OSA, basal angle was the most heritable \((h^2 = 0.61, p = 0.003)\) following the three PF traits discussed earlier (posterior fossa height, PC4, and area5). The 1 LOD down supporting interval for the Chromosome 1 linkage peak spans 15.5 Mb (GRCh37 genomic coordinates: 172810806–188302977) and contains 112 known RefSeq genes (based on the UCSC genome browser; 11/19/2012).

Discussion

We recently completed a whole genome linkage screen using 66 CMI multiplex families in order to identify susceptibility genes (Markunas et al., 2013). The initial analysis using the complete collection of families yielded limited evidence for linkage. Stratified linkage analyses using clinical criteria to reduce potential genetic heterogeneity resulted in increased evidence for linkage to multiple regions of the genome and identification of several biologically plausible candidate genes. Motivated by this approach, we carried out the present study in order to explore the genetic basis of several candidate CMI endophenotypes and the use of these in a stratified linkage analysis in order to identify additional CMI susceptibility genes. Specifically, a heritability analysis was conducted using PF traits and those traits that were found to be both heritable and associated with affection status were used in an OSA.
due to environmental factors. Variation in the degree of CTH appears to be mostly influenced by genetic factors, and much of the PF appears to be strongly influenced by genetic factors and/or environmental exposures could result in a PF that is too small and shallow to accommodate the normal sized cerebellum. Tonsillar herniation may occur subsequently due to additional environmental factors. In support of a genetic contribution to disease in patients that fit under the classical “cranial constriction” mechanism (Milhorat et al., 2010), a substantial portion of the PF showed evidence of being heritable and over half of those traits were associated with disease status ($p < 0.05$) in our families. The most significant finding from the heritability analysis was the PF height ($h^2 = 0.77$). It is also one of the most strongly associated traits with affection status ($p = 0.00002$) and is a good indicator for the overall size of the PF given its strong correlation with the PF area ($p = 0.77$). Importantly, while much of the PF appears to be strongly influenced by genetic factors, variation in the degree of CTH appears to be mostly due to environmental factors ($h^2 = 0$). As stated previously (Boyles et al., 2006), this is consistent with the hypothesis that the primary disease mechanism involves a compromised PF and that tonsillar herniation occurs secondarily. In other words, perturbations during development of the cranial base due to genetic factors and/or environmental exposures could result in a PF that is too small and shallow to accommodate the normal sized cerebellum. Tonsillar herniation may occur subsequently due to additional environmental factors. However, there is accumulating evidence suggesting that tonsillar herniation may not even be necessary for disease [for review see (Markunas et al., 2012)]. Tonsillar herniation does not correlate well with symptoms or provide any information regarding the underlying disease etiology (e.g., due to a brain tumor versus a cramped PF), suggesting that tonsillar herniation alone may not be the best criterion to use for diagnosis.

It is important to note that our heritability estimates differed substantially from the previously published heritabilities estimated for a subset of the PF traits we examined (Boyles et al., 2006). This could be due to several factors including differences in the collection of CMI families used in the analysis (e.g., differences in the age distribution, proportion of males and females, and/or proportion of affected and unaffected individuals), as well as the fact that the most recent heritability analysis controlled for several covariates including age at MRI, sex, affection status, measurer, and ascertainment. Our finding that the clivus was not heritable is, however, somewhat surprising. The lack of significance could be potentially explained if age was not appropriately modeled in our analysis. The clivus is strongly associated with age ($p = 0.002$), consistent with the fact that the sphenoccipital synchondrosis, which is the cartilaginous joint between the basisiocciput and basisphenoid bone does not normally close until ages 16 to 20 (Noudel et al., 2009). However, at this time our sample size is too small to perform an age stratified analysis and explore how heritability of the clivus might vary with respect to age. In addition, as the clivus is comprised of the basisphenoid and the basioccipt bone it could examine these separately rather than collectively in order to refine the analysis.

Several genomic regions were implicated from OSA, including 22q12.3-q13.31 identified within a subset of families characterized by a large PF height, area5, and PC4, all of which are positively correlated with one another. The Chromosome 22 candidate interval contains multiple interesting biological candidates, one of which is a histone acetyltransferase, E1A binding protein p300 (EP300). EP300 and CREB binding protein (CREBBP) act as co-activators of SRY-box containing gene 9 (Sox9), a transcription factor involved in chondrocyte differentiation (Tsuda et al., 2003). Disruption of the CREBBP/EP300/Sox9 complex inhibits collagen, type II, alpha 1 (COL2A1) expression and chondrogenesis (Tsuda et al., 2003), which is an important process preceding formation of the bones in the cranial base (endochondral ossification). In addition, both EP300 and CREBBP have been implicated in Rubenstein-Taybi syndrome (RSTS), which is associated with a wide variety of cranial-cervical and spinal cord complications, many of which share similar clinical features with CMI (Parsley et al., 2011). One of the associated cranial-cervical conditions includes a large foramen magnum

![Figure 3](image)

**Figure 3** Ordered subset analysis multipoint linkage plots. Black lines indicate unconditional multipoint LOD scores, blue lines indicate multipoint LOD scores obtained in the subset of families identified through OSA, and the red dashed line indicates a LOD = 3. (A) Chromosome 22: families were ordered by posterior fossa height in descending order, and (B) Chromosome 1: families were ordered by basal angle in ascending order.
that is consistent with how this linkage interval was identified. The subset of families showing maximal evidence for linkage to this region were defined by a large PF height which is positively correlated with the width of the foramen magnum ($\rho = 0.19, p$-value $= 0.03$). In addition, RSTS and Chiari Malformation have been reported to co-occur (Wojcik et al., 2010; Kim et al., 2010; Parsley et al., 2011). Interestingly, within a morphometrically similar subset of families (large PF height) we observed a slight increase in evidence for linkage to a region containing the related gene, CREBBP ($N = 8$ families, Max LOD $= 1.9, \Delta$LOD $= 1.87$, emp $p$-value $= 0.05$). In addition to EP300, another gene of interest within the candidate interval on chromosome 22 is the activating transcription factor 4 (ATF4), which interacts with EP300 and CREBBP. ATF4 plays a major role in the regulation of osteoblast differentiation and function (Liu & Lee, 2012), an important process in cranial base bone formation. Interestingly, activating transcription factor 3 (ATF3), which plays a role in the terminal differentiation of chondrocytes (James et al., 2006), is present within a previously identified candidate linkage interval on chromosome 1 (Max LOD $= 2.3$) identified in families with a history of connective tissue disorder (CTD) related conditions (Markunas et al., 2013).

In addition to the Chromosome 22 candidate interval, a significant linkage peak was identified on Chromosome 1 (1q24.3-q31.1) using a subset of families characterized by small basal angles. This region contains 112 known genes; one of particular interest is LIM homeobox 4 ($LHX4$). $LHX4$ is a transcription factor involved in regulating the proliferation and differentiation of pituitary cell lineages (Sheng et al., 1997), mutations in which have been identified in patients with pituitary hormone deficiencies (Machinis et al., 2001, Tajima et al., 2007). Interestingly, there has been one report of a patient with a de novo missense mutation in $LHX4$, severe combined pituitary hormone deficiency (CPHD), a small sella turcica, and CMI (Tajima et al., 2007), as well as an additional report of a family that showed segregation of a splice site mutation in $LHX4$ with pituitary problems, pointed cerebellar tonsils (a characteristic shared with some CMI patients), and a poorly developed sella turcica (Machinis et al., 2001). Of further interest is the potential link between the sella turcica and the basal angle that was used to identify the subset of families that showed increased evidence for linkage to this region on Chromosome 1. Since the basal angle is measured as the angle between lines extending out from the center of the sella turcica to the nasion and basion, it would seem plausible that the small basal angles identified in this subset of families could be at least partially due to an altered sella turcica. However, further radiological work would be needed to truly assess this hypothesis.

Although we identified several plausible candidate genes by restricting our linkage analysis to subsets of families that were similar with respect to heritable, disease-relevant cranial base morphological traits, there are several important study limitations to consider. First, our sample size for the analyses was relatively small. Out of the 66 families included in our primary whole genome linkage screen (Markunas et al., 2013), only 49 families were useful for OSA due to the limited availability of MRIs. In addition, out of those 49 families every family did not contain more than one affected family member with an MRI available thus the family-level covariate values used for OSA may be strongly influenced by outliers. While this may result in reduced power, we did identify several regions of interest including one region on Chromosome 22 that remained significant even after a conservative bonferroni correction for multiple testing.

Both genetic and environmental factors contribute to variation in the cranial base and thus influence risk for CMI. Importantly, tonsillar herniation, the gold standard by which individuals are diagnosed, was not found to be heritable lending further support to the hypothesis that tonsillar herniation may not be the best criterion to use for diagnosis as it likely occurs secondarily, does not correlate well with symptoms, and may not be necessary to cause disease. Future studies, particularly genetic studies, should explore the use of additional heritable, disease-relevant traits, such as PF height. Although we were underpowered to perform a whole genome quantitative screen, PF traits were used in stratified linkage analyses yielding several plausible candidate genes including EP300, CREBBP, ATF4, and $LHX4$ which are currently being sequenced in our families. Approaching CMI as an etiologically heterogeneous disease and using heritable, disease-relevant PF traits rather than CTH solely has the potential to yield promising results in future genetic studies.

Acknowledgements

We would like to thank all family members for participating in the Chiari genetics study. Financial support for this study was generously provided by the National Institutes of Health (NS063273, AAK and SGG), American Syringomyelia and Chiari Alliance Project (AAK), and Chiari and Syringomyelia Foundation (CAM). We have read the journal’s policy and have the following conflict of interest: Dr. Allison Ashley-Koch is chair of the Chiari and Syringomyelia Foundation (CSF) scientific, education, and advisory board. CSF provided partial funding for this study, as well as salary support for Dr. Christina Markunas. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
References


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1** Pearson correlation matrix of posterior fossa traits.

*Received: 4 April 2013  
Accepted: 21 August 2013*
**Medical By-Laws**  
**Scientific Educational & Advisory Board (SEAB)**

**Article 1 – Purpose**  
The purpose of the SEAB is to advise the CSF Board of Directors, with a diversity of perspectives, on matters relating to current clinical treatment and scientific research, and to provide peer reviewed educational material for individuals affected by Chiari Malformation, syringomyelia and related Central Nervous System (CNS) disorders.

**Article 2 – Membership**  
1. The SEAB shall consist of an Executive Committee and a general Scientific, Educational and Advisory Board.

2. Number of Members: The size of the SEAB may vary from time to time. There will be an Executive Committee of seven members. However, the maximum number of medical, scientific and professional members for Executive Committee and SEAB members shall be twenty-two in total.

3. Terms of Appointment:  
   - Officers:
     - Chairman shall be appointed for a two-year term.
     - Vice-Chairman shall be appointed for a two-year term and then serve as the Chairman.
   - Executive Committee Members at Large:
     - Executive Committee Members shall have a three-year term and may be reappointed for an additional term of three years. This is independent of SEAB membership terms.
   - SEAB Members:
     - Members of the SEAB will serve for a term of three years and may be reappointed, upon request
     - After two consecutive terms, the member must leave the board and, upon request, can be reappointed after one year
     - Terms of members will be staggered so that less than half of the members’ terms expire in any one-year.

4. Nomination and Appointment: Candidates may be nominated by a member of the Medical Research Board or by the Board of Directors. The Board of Directors is
5. Selection Criteria: According to the CSF By-Laws, the SEAB must include a diverse group of medical, scientific and other professionals, who are interested in research or providing medical care to patients diagnosed with CM/SM and related disorders. Whenever possible, the SEAB shall include the perspectives of providers who are actively involved in specialty care (e.g., neurology, neurosurgery, orthopedics, physiatry, occupational medicine), primary care (e.g., family practice), ancillary care (e.g., physical therapy), veterinary medicine, basic science and research professionals. In addition to their ability to represent the perspective of their profession, members will be selected for their ability to represent the interests of the community at large.

6. Resignations and Replacement Appointment: If a member finds it necessary to resign from the SEAB, they are encouraged to remain until a replacement can be selected and to provide as much notice as possible. They are also encouraged to help the SEAB find a suitable replacement. Replacement members will be appointed to the remainder of the resigning member's term, and are eligible for reappointment at the discretion of the Board of Directors. After service on the SEAB, all members of SEAB will have a lifetime membership on the Senior Advisory Panel.

7. Duties of Members: Regular attendance is vital to the purposes of the SEAB. Members accept the duty and obligation to attend meetings and to provide advance notice if they are unable to attend. Repeated absences may be considered an abdication of the appointment, and may be grounds for terminating a member's appointment at the discretion of the Board of Directors, with the recommendation of the Executive Committee. SEAB members are encouraged to participate in local chapters or consider helping to form one in their area.

8. Self-Promotion: Chapters shall not be used as a vehicle for self-promotion. Medical professionals should exhibit ethical behavior and not solicit patients directly or indirectly. Medical professionals will restrict the motive of personal gain and stress the necessity of service through education and awareness. See Conflict of Interest Policy.

Article 3 – Executive Committee
1. Officers: Officers of the Executive Committee are Chairman, Vice-Chairman and five members at large.

2. Selection: Officers will be elected by a majority of members of the SEAB and ratified by the Board of Directors.

3. Terms of Office: Elections shall be held every two years or at the request of a majority of the members of the SEAB. Each Officer shall serve until a replacement is selected.
4. Duties and Responsibilities: The Officers are to set the agenda for each meeting, run
the meetings, maintain a liaison with the Board of Directors, offer to the Board of
Directors the Executive Committee's advice on medical issues, and forward formal
recommendations and opinions to the Board of Directors.

**Article 4 – Subcommittees**
1. Special Committees: Special committees may be formed from time to time by the
Executive Committee.

2. Powers, Duties and Responsibilities: Powers, duties and responsibilities of
subcommittees shall be as assigned by the Executive Committee.

3. Membership: Non-members of the SEAB may serve on subcommittees at the
consent of the full SEAB.

4. Authority: The authority of any subcommittee is to advise the full SEAB on issues as
assigned.

**Article 5 – Order of Business and Schedule of Meetings**
1. Meeting Schedule: Meetings will be held at least three times annually by conference
call and one in conjunction with a medical conference, as scheduled by the SEAB
Chairman.

2. Agenda Development: The agenda for the next meeting will be developed by the
Chairman and/or Vice-Chairman of the Executive Committee, with the assistance of
CSF staff. Any member wishing to include an item on the agenda has the responsibility
to draft and present the agenda item to the Chairman for approval and inclusion.

3. Agenda Distribution: The agenda will be published by CSF staff and distributed to
members at least one week prior to the next meeting.

4. Meeting Records: Staff from CSF will tape meeting proceedings and will prepare
meeting notes for approval by the Chairman and Vice Chairman and distribution to the
members prior to the next meeting.

**Article 6 – Parliamentary Authority**
1. Establishing a Majority: For administrative decisions, such as election of Officers,
recommendations to remove a member, or changing the by-laws, a majority is
established by a majority of all members of the SEAB. For other matters, a majority is
established by a simple majority of all members present.

2. Advising the Board of Directors: The Executive Committee will vote on any issue
requiring advice to the Board of Directors. Consensus is not required. CSF staff will
assist the Executive Committee in writing up the Executive Committee's advice and
presenting it to the Board of Directors.
3. Minority Reports: On any issue resulting in advice to the Board of Directors, if there is not consensus among all members of the Executive Committee, minority members are encouraged to submit minority reports for the Board of Directors’ consideration.

Article 7 – Amendment Procedures
Any articles of these by-laws may be added, deleted or amended by a majority vote of at least two thirds of the membership of the Executive Committee and must be ratified by the Board of Directors.
SEA Board Rotation

- Dr. Allison Ashley-Koch to turn SEA Board Chair to Dr. Rick Batzdorf (2 year term) at the end of the meeting

- Dr. Allison Ashley-Koch will move to the Senior Advisory Panel

- Elect a new Vice-Chairman for 2 year term from the members of Executive Committee listed below:

  Dr. Richard Ellenbogen  3 year term  2015
  Dr. Dominic Marino*  3 year term  2015
  Dr. Harold Rekate  3 year term  2015
  Dr. Clair Francomano  3 year term  2015
  Dr. Fraser Henderson  3 year term  2015

  *Dr. Ashley-Koch and Dr. Batzdorf have nominated
  Dr. Dominic Marino for this position

- Elect a New Executive Committee member for a 3 year term
  Dr. Ashley-Koch and Dr. Batzdorf have nominated
  Dr. Mark Luciano for Executive Committee (3 year term)

- Reappointment of SEA Board members:
  Dr. John Oro has requested appointment for his second three year term

- Nomination & election of three new SEA Board members
CONFLICT OF INTEREST POLICY
CHIARI & SYRINGOMYELIA FOUNDATION BOARDS / COMMITTEES

I. PURPOSE

The purpose of this Chiari Syringomyelia Foundation (CSF) Conflict of Interest Policy is to safeguard the integrity and independence of the Chiari Syringomyelia Foundation’s Boards by educating Chiari Syringomyelia Foundation Board Members regarding situations that generate actual, potential, or perceived Conflicts of Interest and providing procedures for Board Members to disclose, and for the Board of Directors and the Executive Committee to address and resolve, such Conflicts. CSF Board Members are entrusted to promote the CSF mission and to act without personal, professional, or financial interest or benefit. Accordingly, no Member may use his or her position on the CSF Board for personal, professional, or financial gain or to benefit another at the expense of the CSF mission or reputation.

II. DEFINITIONS

A. INTERESTED PERSON

“Interested Person” means any Chiari Syringomyelia Foundation Board Member/Committee Member who has a direct or indirect Secondary Interest, whether personally or through business, investment, or immediate family.

B. SECONDARY INTEREST

“Secondary Interest” means:

1. Any actual or potential ownership or investment interest in, or compensation arrangement or other financial affiliation with, any entity or individual:

   (i) That is subject to review or analysis by the Chiari Syringomyelia Foundation Board.

   (ii) With which the Chiari Syringomyelia Foundation has, or is negotiating, a transaction or arrangement; or

2. Any non-remunerative activity engaged in by a Board Member which may have the potential of creating bias in the Board Member including, without limitation, faculty positions, service on an Advisory Board, and academic and/or professional research.
C. **COMPENSATION**

“Compensation” includes both direct and indirect remuneration.

D. **CONFLICT OF INTEREST**

A “Conflict of Interest” exists when, in the judgment of the COI Subcommittee, and Chiari Syringomyelia Foundation Board of Directors’ judgment concerning the promotion of the CSF mission has the potential to be unduly influenced by a Secondary Interest.

A Secondary Interest is not necessarily a Conflict of Interest. Under Section IV.C, a person who has a Secondary Interest may have a Conflict of Interest only if the COI Subcommittee determines that a Conflict of Interest exists.

E. **CSF**

“CSF” means the Chiari Syringomyelia Foundation and/or any of its Boards.

F. **CSF BOARD/COMMITTEE MEMBER**

“CSF Board /Committee Member” means a member of the Chiari Syringomyelia Foundation Board / Committees and/or any of its subcommittees, or any person affiliated with either in a consulting, advisory, or other capacity.

G. **COI SUBCOMMITTEE**

“COI Subcommittee” or “Subcommittee” means the Conflict of Interest Subcommittee responsible for determining whether a Conflict of Interest exists. The Chiari Syringomyelia Foundation Board Chairpersons shall appoint the three-person COI Subcommittee, and may not serve on the Subcommittee. A Member who has been appointed to the COI Subcommittee and whose Secondary Interest subsequently becomes subject to the COI Subcommittee’s review, will recuse him or herself from the review of his or her own Secondary Interest and will be replaced by another Member of the Board at the discretion of the Chairpersons.

III. **ELIGIBILITY**

A. A person shall be eligible to serve as a Chiari Syringomyelia Foundation Board Member if the person and the person’s immediate family members:

1. Do not have an ownership or investment interest in, or compensation arrangement or other financial affiliation with, the Chiari Syringomyelia Foundation.
2. Are not employees of the Chiari Syringomyelia Foundation.

3. Do not directly or indirectly have a business relationship with the Chiari Syringomyelia Foundation with which the relationship might affect the person’s independence in decision-making.

Notwithstanding anything to the contrary above and without affecting eligibility, Chiari Syringomyelia Foundation Board Members are entitled to reimbursement from the Chiari Syringomyelia Foundation for reasonable travel and related expenses incurred in connection with such Board Members’ service on the Chiari Syringomyelia Foundation Board, Executive Committee, Committee, or Subcommittee.

B. To accept the position of Board of Directors member, Board of Trustee member, or member of the Executive Committee of the SEAB, the Board of Directors asks that this person resign from the Board of any related organization, to devote his/her time to the Chiari & Syringomyelia Foundation.

IV. PROCEDURES

A. DUTY TO DISCLOSE

An Interested Person must disclose to the COI Subcommittee the existence of any Secondary Interest of which he or she is aware, or becomes aware during the course of his or her Chiari Syringomyelia Foundation Board service, and must disclose to the COI Subcommittee all material facts related thereto.

B. RECUSAL OF SELF

Any Member may recuse himself or herself at any time from involvement in any decision or discussion in which the COI Subcommittee believes he or she has or may have a Conflict of Interest, without determination of whether a Conflict of Interest exists.

C. DETERMINING WHETHER A CONFLICT OF INTEREST EXISTS

After disclosure by the Interested Person of the Secondary Interest and all material facts concerning such Secondary Interest, he or she shall retire from the room, and the COI Subcommittee shall meet to discuss and determine whether a Conflict of Interest exists.

The COI Subcommittee shall review all disclosures and related materials submitted by the Interested Person, and any other materials the Subcommittee deems relevant, and render a reasoned decision as to whether the Secondary Interest could directly and significantly affect the Interested Person’s ability to maintain unbiased, ethical interactions with the Chiari Syringomyelia Foundation and/or advance the Chiari Syringomyelia Foundation’s mission. If a Conflict of Interest is deemed to exist, the COI Subcommittee will work with the Interested Person to manage, minimize, or eliminate the Conflict of Interest.
D. ADDRESSING THE CONFLICT OF INTEREST

1. If the COI Subcommittee determines that a Conflict of Interest exists, the Interested Person may not participate in, and must abstain from, all deliberations with regard to the subject matter, transaction, or arrangement in which he or she has a Secondary Interest, unless he or she has special information of a technical nature that will help the Chiari Syringomyelia Foundation Boards better understand a particular issue. In that case, the Interested Person will be allowed to supply such information before recusing himself or herself from the deliberations, but may not participate in, and must abstain from, all decision-making with regard to the subject matter, transaction, or arrangement in which he or she has a Secondary Interest.

2. The remaining Chiari Syringomyelia Foundation Board Members may approve a transaction or arrangement, if those Members determine the transaction or arrangement:

   (i) Promotes the Chiari Syringomyelia Foundation’s mission;

   (ii) Is fair and reasonable; and

   (iii) Is the most advantageous transaction or arrangement the Chiari Syringomyelia Foundation can obtain with reasonable efforts under the circumstances.

E. VIOLATIONS OF CONFLICT OF INTEREST POLICY

1. If the COI Subcommittee has reasonable cause to believe a Member has failed to disclose actual or possible Conflicts of Interest, the Subcommittee shall investigate the potential Conflict of Interest violation and shall inform such Member of the basis for such belief and afford him or her an opportunity to explain the alleged failure to disclose.

2. If, after hearing the response of such Member and after making further investigation as warranted by the circumstances, the COI Subcommittee determines that the Member has failed to disclose an actual or possible Conflict of Interest, it shall take appropriate disciplinary and corrective action. Such action may include formal reprimand, cancellation of the transaction or arrangement generating the Conflict of Interest, suspension from the Chiari Syringomyelia Foundation Board, and/or removal from the Chiari Syringomyelia Foundation Board.

V. CONSULTANTS AND EXPERTS

All consultants and experts retained by the Chiari Syringomyelia Foundation or on its behalf for a specified assignment are subject to the eligibility requirements and Conflict
of Interest procedures set forth in Sections III and IV, respectively. In addition, all consultants and experts retained by the Chiari Syringomyelia Foundation may not have any ownership or investment interest in, or compensation arrangement or other financial affiliation with, any individual or entity that is materially related to the subject matter of the consultant or expert’s specified assignment, or any other personal, professional, or financial interest that would affect the consultant or expert’s independence in decision-making.

Notwithstanding anything to the contrary and without affecting eligibility, consultants and experts retained by the Chiari Syringomyelia Foundation or on its behalf shall be entitled to compensation by the Chiari Syringomyelia Foundation at their standard hourly rates for services performed in connection with such assignment, or on such other basis as may have been agreed to by, and disclosed to, the Boards.

VI. ADDITIONAL RULES OF CONDUCT/CONFIDENTIALITY

In addition to complying with the procedures described above, Chiari Syringomyelia Foundation Board Members shall take the following steps to safeguard the integrity and reputation of the CSF Boards/Subcommittee:

1. All oral and/or written communications between and/or among the Chiari Syringomyelia Foundation Board Members, CSF Committee agents, and/or the CSF and its representatives (including legal counsel); all work product of the CSF Boards/Committees (other than communications or work product expressly intended by the CSF Boards/Committees and the CSF for public disclosure or otherwise in the public record); and all other non-public information (including, without limitation, “Data,” as that term is defined in Section III.A of the CSF Committee Data Policy) entrusted to or obtained by a Member by reason of his or her position as a Member (together, the “Confidential Information”) shall be treated by Members as confidential, shall be treated in accordance with any and all applicable privileges, including the attorney-client privilege, and shall not be used by any Member for his or her own personal benefit or to benefit persons or entities outside the CSF Boards/Committees;

2. All oral and/or written communications between and/or among the Chiari Syringomyelia Foundation Board/Committee Members, CSF Committee agents, and/or the CSF and its representatives, shall be conducted in a professional, courteous, and respectful manner; and

3. No Chiari Syringomyelia Foundation Board/Committee Member shall use his or her status as a Member in any manner that is inconsistent with, or adverse to, the CSF’s mission. This prohibition includes, without limitation, engaging in media-related activities or other forms of self-promotion that either involve the disclosure of Confidential Information or are otherwise inconsistent with, or adverse to, the CSF’s mission or any work or research being conducted by the CSF Boards/Committees.
VII: DISTRIBUTION OF CONFLICT OF INTEREST POLICY

Each Chiari Syringomyelia Foundation Committee Member shall **annually** sign a statement that affirms that such Board/Committee Member:

1. Has received a copy of the Conflict of Interest Policy;
2. Has read and understands the Policy; and
3. Has agreed to comply with the Policy.

CERTIFICATION OF COMPLIANCE
WITH CONFLICT OF INTEREST POLICY

I, ______________________, affirm that I have received a copy of the Conflict of Interest Policy for the Chiari Syringomyelia Foundation and have read and understand the Policy, and agree to be bound by, and comply with the Policy, effective as of the date of my commencement of service on the Chiari Syringomyelia Foundation Board/Committee.

_________________________  ______________________
Signature                          Date

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Revision Date: 3.29.2011
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# Meeting Teleconference Attendee Contact Info

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The Participant shall protect the disclosed confidential information by using the same degree of care, but no less than a reasonable degree of care, to prevent the unauthorized use, dissemination, or publication of the confidential information as the Participant uses to protect its own confidential information of a like nature.