Current Findings in Genetics of Chiari Type I Malformation

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Outline

- Background
  - Evidence for a genetic component
  - Research challenges
  - Previous findings

- Current Findings
  - Targeted NextGen Sequencing of Candidate Genes

- Concluding remarks
  - Summary of what we learned
  - Future directions
Why genetics is important for CMI

- Precision Medicine
  - Predictive power—who is at risk?
  - Prognostic value—who is going to develop symptoms?
  - Therapeutic response—who is going to respond better or worse to particular treatments?
Support for a genetic contribution to CMI

- Familial aggregation
- Twin studies
- Co-occurrence with known genetic syndromes
Research challenges

- Difficult to ascertain a large collection of families with multiple individuals affected
  - Relatively rare condition
  - Minority of cases known to be familial

- Challenges in defining who meets criteria for CMI
  - No consensus diagnostic criteria
  - Tonsillar herniation does not correlate well with symptom presentation
Complex etiology: genetic and environmental factors

- **Background**
  - Next generation sequencing of candidate genes
  - Concluding remarks

- **Evidence for a genetic component**

- **Research challenges**

- **Previous findings**

- **Complex etiology: genetic and environmental factors**

- **CMI**
  - Cranial Constriction
  - Intraspinal Hypotension
  - Intracranial Hypertension

- **Spinal Cord Tethering**
  - Intracranial Hypertension

- **G1**, **G2**, **E1**, **E2**, **G3**

- **Cranial Settling**
Accumulating evidence supports an association between hereditary connective tissue disorders (CTDs) and CMI.

CMI patients diagnosed with CTDs may represent a distinct class of patients.

- Occipital bone and PF volume are expected size but craniocervical instability exists.
- This is in contrast to the smaller occipital bones and PF volume observed in “classical” CMI patients believed to have a “cranial constriction” etiologic mechanism.
Genetic Dogma for Chiari Malformations

1. DNA
2. RNA
3. Protein
4. Morphologic Traits
5. Chiari Malformation
Genome-wide linkage screen to identify CMI genes

Markunas et al., 2013a

- Genome-wide screen of 66 families for CMI
- Genotyped over 500,000 SNPs
- Stratified families on presence or absence of connective tissue disorder symptoms
- Identified mutations in GDF6, a gene associated with Klippel-Feil Syndrome, in CTD- families
- Several other genomic regions provided some evidence for association
Candidate gene study of CMI and posterior fossa morphology

Urbizu et al., 2014

- Selected 58 genes involved in forming the occipital somites which ultimately form the posterior part of the skull
- Compared common genetic variants among cases with CMI versus controls and also looked at the association with cranial morphology
Candidate gene Next Gen sequencing

- 21 genes prioritized from previous work

- Identify possible genetic changes that are functional that may be associated with CMI and cranial morphology

- Determine the relationship between these genes and patients with and without CTD
CMI cases were identified from the Chiari1000 project (n=94) and the Duke genetic project (n=92)

- All female and NHW
- Wide age range (10 to 82 years old)

Everyone consented and provided a genetic sample, as well as clinical information

We defined CTD status based on the presence of a Beighton score and symptoms:

- Hypermobility
- Mitral valve prolapse
- Aneurysm
- Kyphosis
## Data Set

<table>
<thead>
<tr>
<th></th>
<th>Chiari 1000</th>
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<th>Duke</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>CTD+</td>
<td>28</td>
<td>29.79</td>
<td>53</td>
<td>57.61</td>
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<tr>
<td>CTD-</td>
<td>66</td>
<td>70.21</td>
<td>27</td>
<td>29.35</td>
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<td>13.04</td>
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<td>EDS-</td>
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<td>EDS+</td>
<td>5</td>
<td>5.32</td>
<td>5</td>
<td>5.43</td>
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<tr>
<td>Syringomyelia</td>
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<td>14.89</td>
<td>22</td>
<td>23.91</td>
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<tr>
<td>No syringomyelia</td>
<td>80</td>
<td>85.11</td>
<td>70</td>
<td>76.09</td>
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</table>
Targeted Genomic NextGen Sequencing

- Experiment designed to capture exonic (protein coding) regions of the 21 candidate genes

- Analyzed the data to identify variants in CMI patients that were not present, or rarely present, in individuals without CMI
  - Public data from the gnomAD non-Finnish European database (55,860 exomes + 7,509 genomes)

- Compared the number of rare, functional variants in CMI vs controls by gene

- Also compared variants in CTD+ vs CTD- CMI patients

- Using another sequencing technology to confirm variants
Overview of Sequencing Results

- We identified 1345 total variants in the 21 genes in our cohort
  - 777 exonic variants, of which 489 were functional
  - Most were common and present in public databases and unlikely to be associated with CMI
Gene-based tests for rare variants

Were the number of functional variants in the genes different among CMI patients vs controls?

<table>
<thead>
<tr>
<th>Gene</th>
<th>P-value</th>
<th>Odds Ratio</th>
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<tbody>
<tr>
<td>COL5A2</td>
<td>0.0001</td>
<td>1.857</td>
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<tr>
<td>COL7A1</td>
<td>&lt;0.0001</td>
<td>3.191</td>
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<tr>
<td>COL1A2</td>
<td>0.0095</td>
<td>8.273</td>
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<tr>
<td>NRP1</td>
<td>0.0013</td>
<td>50.975</td>
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<tr>
<td>VEGFB</td>
<td>0.0036</td>
<td>7.436</td>
</tr>
<tr>
<td>FLT1</td>
<td>0.0003</td>
<td>3.656</td>
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</table>
Gene-based tests for rare variants

Were the number of variants in the genes different among patients with and without CTD?

<table>
<thead>
<tr>
<th>Gene</th>
<th>P-value</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL7A1</td>
<td>0.028</td>
<td>4.55</td>
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<td>CDX1</td>
<td>0.016</td>
<td>3.86</td>
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<td>VEGFA</td>
<td>0.001</td>
<td>6.65</td>
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<tr>
<td>DSE</td>
<td>0.037</td>
<td>3.45</td>
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</table>
What do these genes have to do with Chiari?

- **COL5A2**
  - Previously associated with EDS
  - Expressed in bone

- **COL7A1**
  - Previously associated with Epidermolysis Bullosa and osteoporosis
  - Highly expressed in skin, but also many other tissues including spinal cord and brain

- **COL1A2**
  - Previously associated with EDS, Osteogenesis Imperfecta and osteoporosis
  - Expressed in many different tissues, including neurologic
What do these genes have to do with Chiari?

- NRP1, FLT1, VEGFA and VEGFB
  - Part of the VEGF pathway which is a growth signaling pathway
  - Important for placental development during pregnancy and for vascular development in general
NextGen Sequencing: Summary

- There continues to be support for many genes being involved in risk for CMI

- CTD status likely is related to the different genes that are involved

- Genes involved in collagen and in the VEGF pathway are strong candidates
Concluding remarks

- The biologic mechanisms causing CMI are primarily developmental and very complicated, but we are making progress towards identifying the key genetic players.

- Ultimately this information could help us diagnose folks earlier and perhaps even determine their prognosis and response to certain interventions/surgeries.

- There is still much work to be done!
Next step is to look at the same genes to see if they are associated with cranial morphometric traits.

Ultimately we hope to expand our search to more patients and more genes.
Acknowledgements

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  - Dorothy Loth

- All study participants