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Current Findings in Genetics of Chiari Type I Malformation

Allison Ashley-Koch, Ph.D.

Professor, Departments of Medicine, Biostatistics & Bioinformatics, and Molecular Genetics & Microbiology

Duke University Medical Center

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?

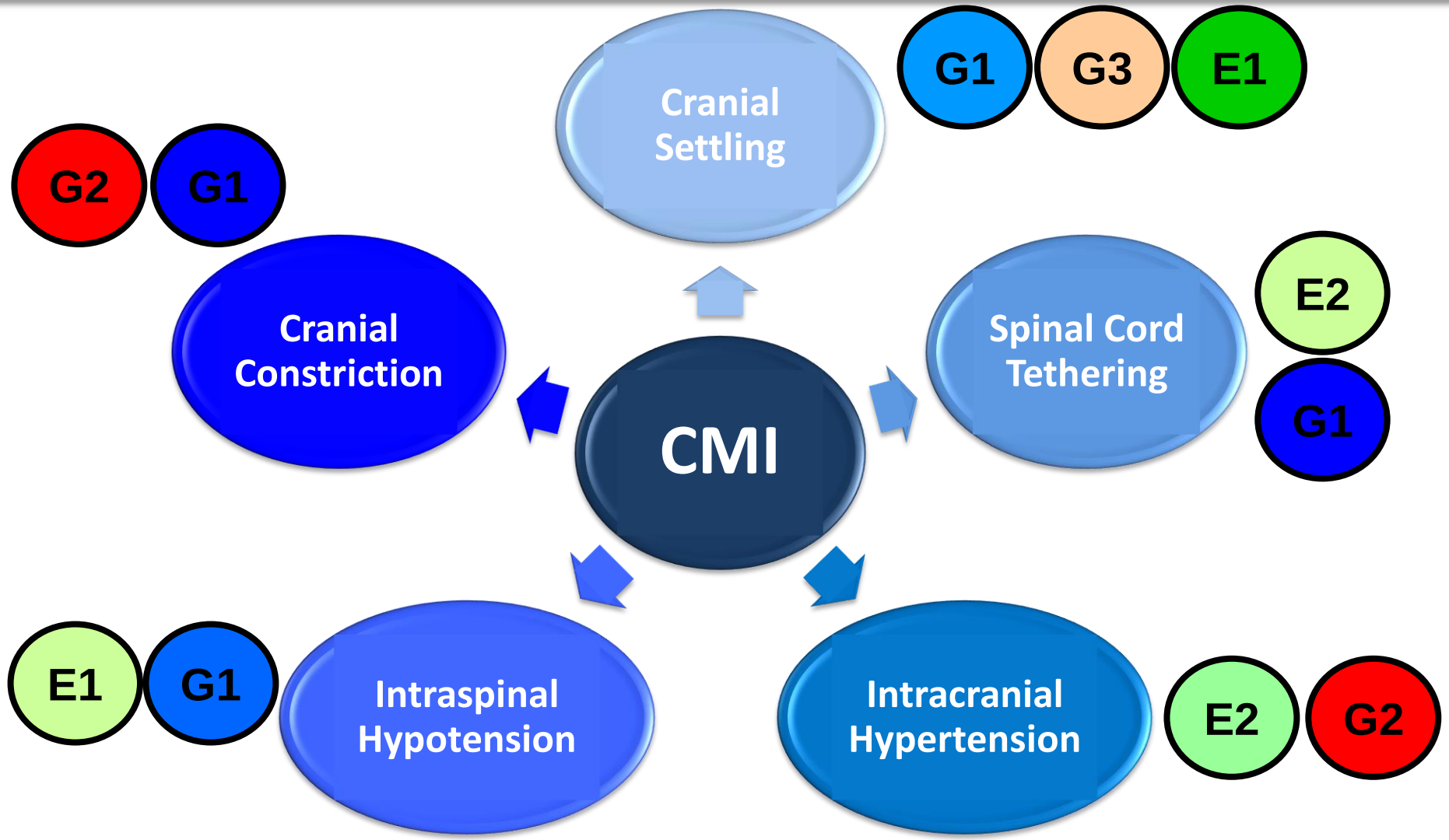
Support for a genetic contribution to CMI

- 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

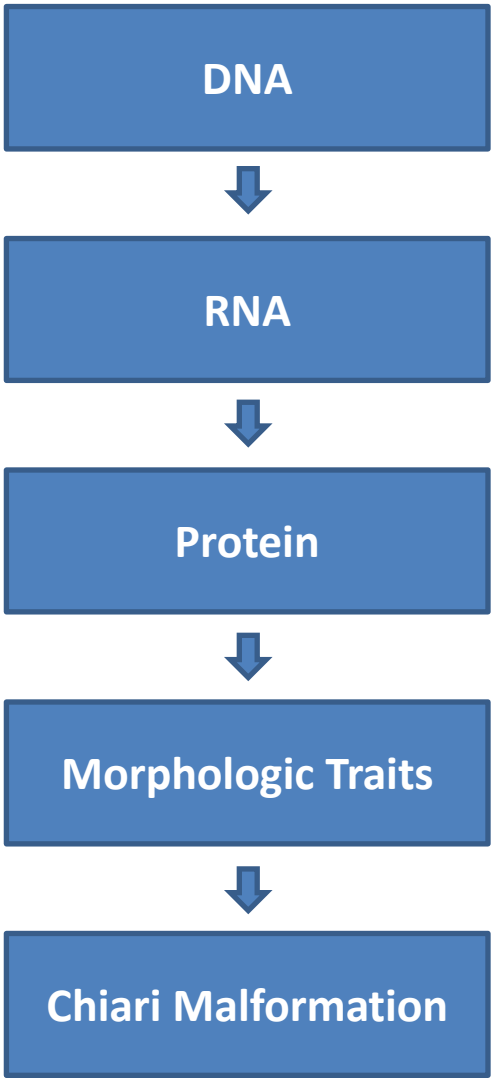


Clinical heterogeneity

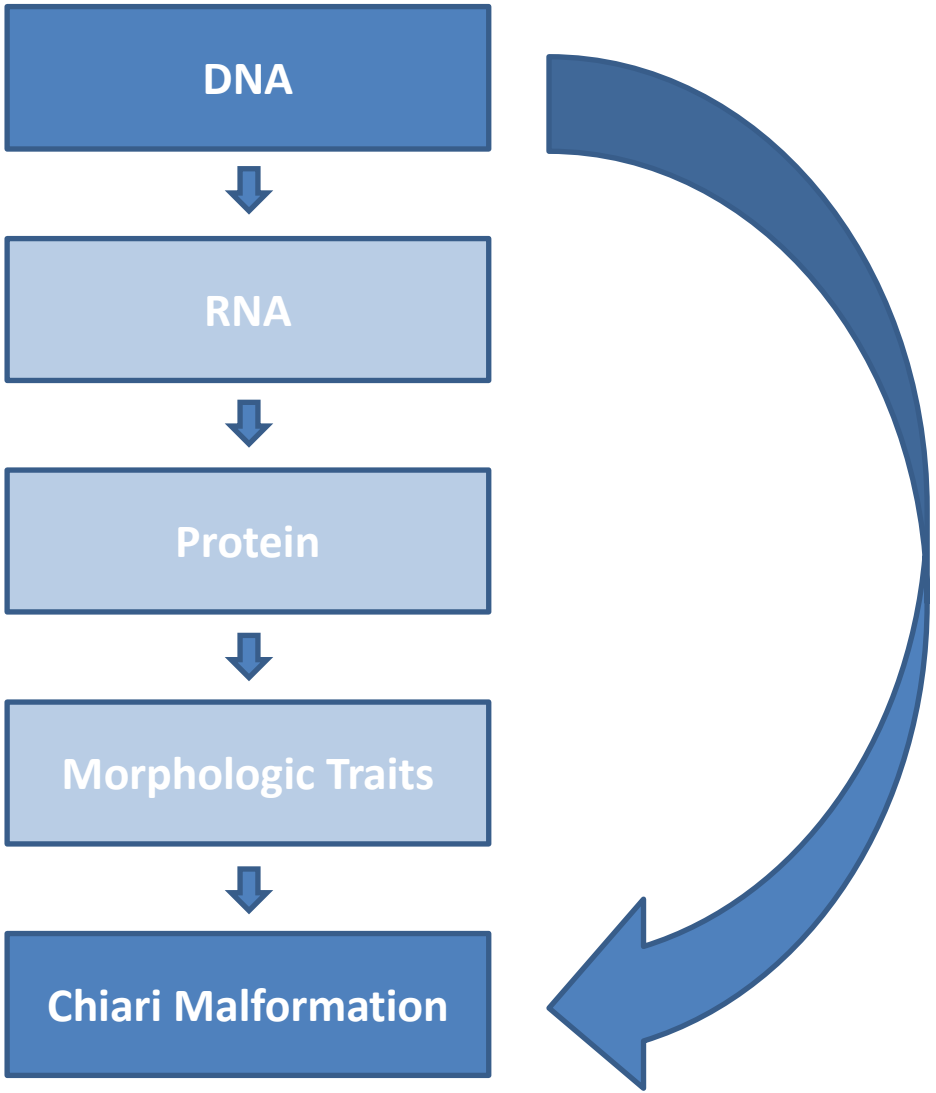
- Accumulating evidence supports an association between hereditary connective tissue disorders (CTDs) and CMI

- 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



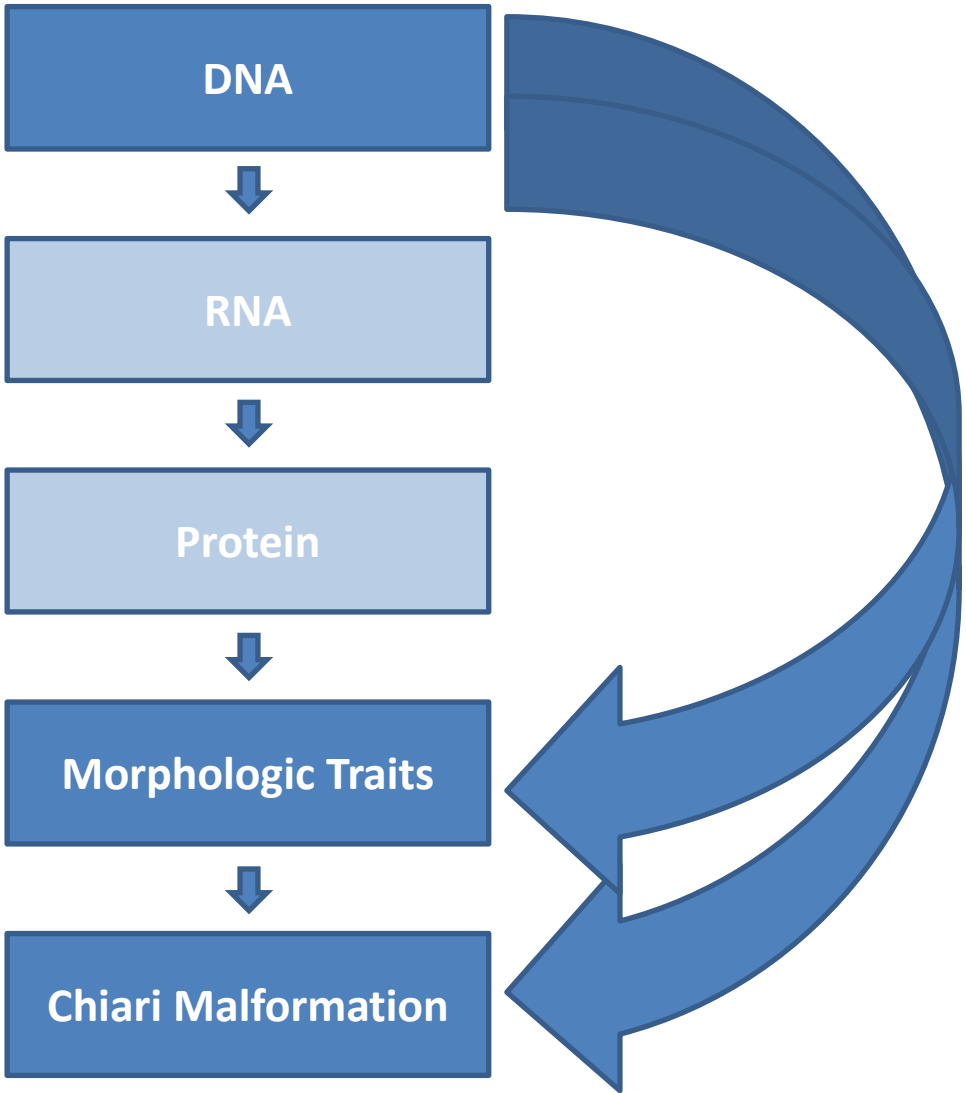
Genome-wide linkage screen to identify CMI genes



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

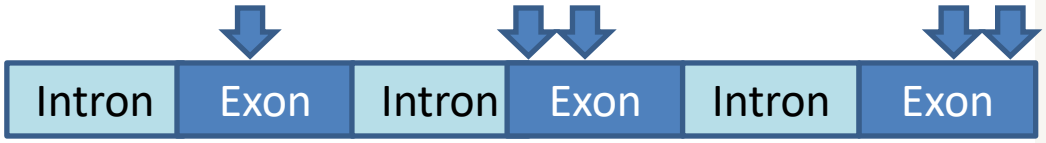


Urbizu et al., 2014

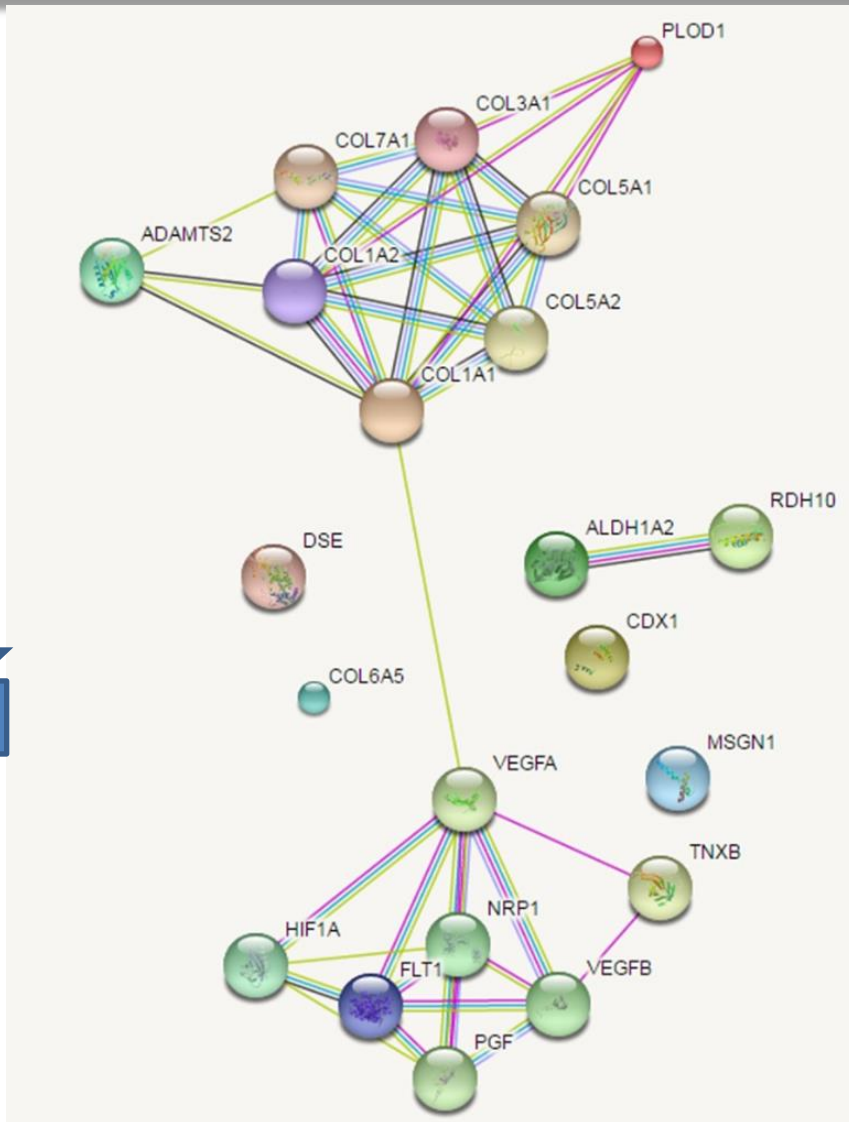
- Selected 58 genes involved in forming the occipital somites which ultimately form the posterior part of the skull
- Compared common genetic variants among cases with CMI versus controls and also looked at the association with cranial morphology

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



Data Set

- 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

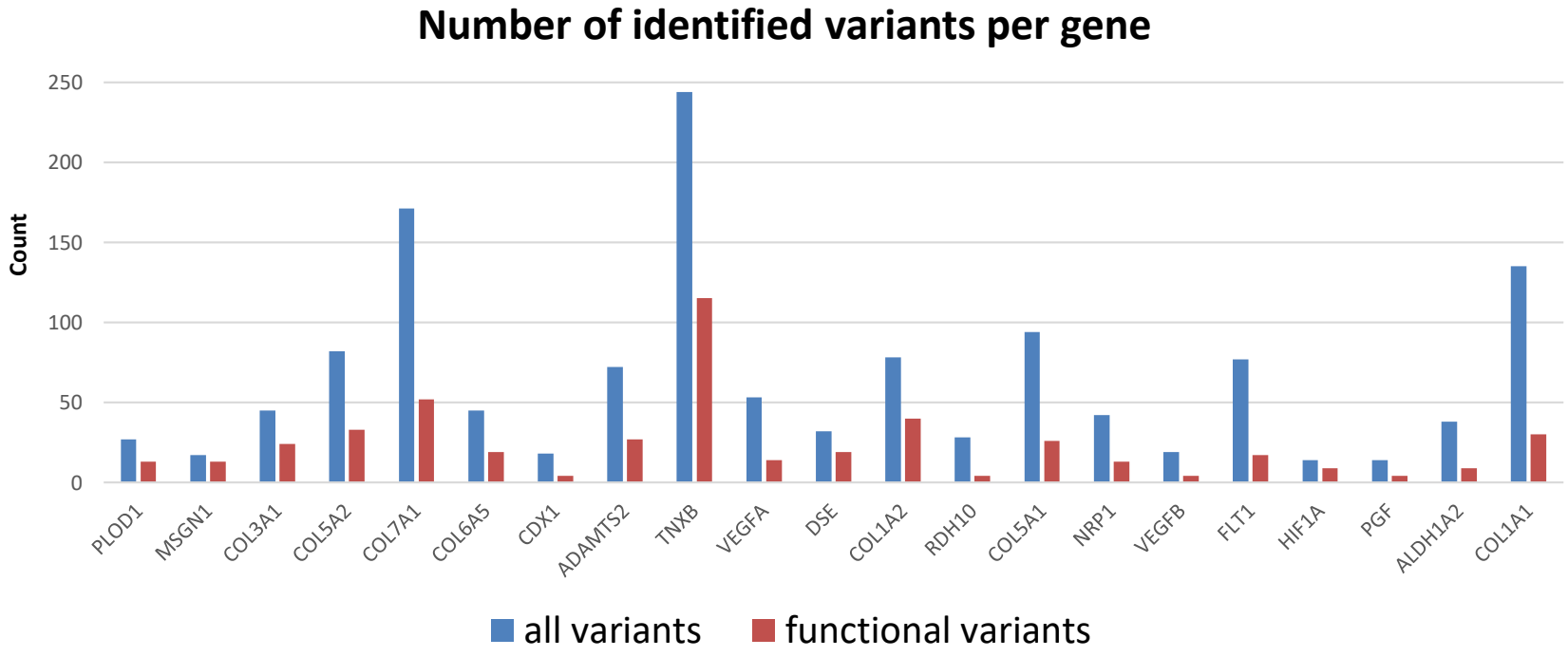
	Chiari 1000		Duke	
	Number	%	Number	%
CTD+	28	29.79	53	57.61
CTD-	66	70.21	27	29.35
Unknown CTD			12	13.04
EDS-	89	94.68	87	94.57
EDS+	5	5.32	5	5.43
Syringomyelia	14	14.89	22	23.91
No syringomyelia	80	85.11	70	76.09

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?

Gene	P-value	Odds Ratio
COL5A2	0.0001	1.857
COL7A1	<0.0001	3.191
COL1A2	0.0095	8.273
NRP1	0.0013	50.975
VEGFB	0.0036	7.436
FLT1	0.0003	3.656

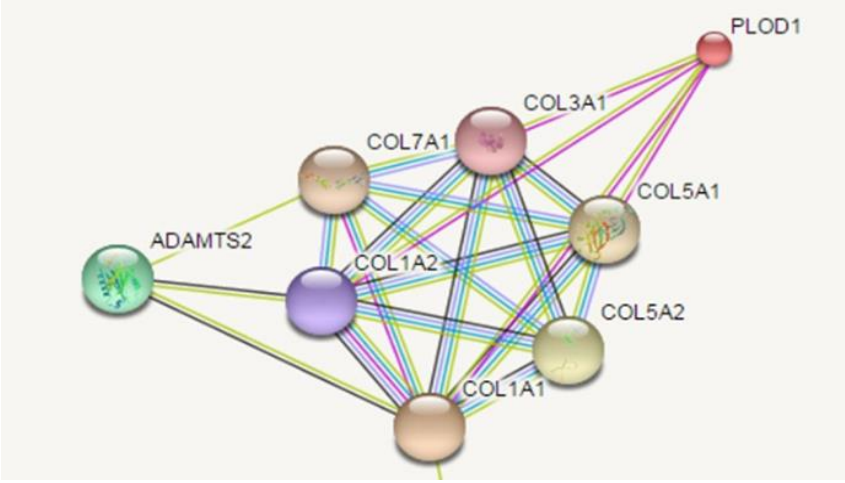
Gene-based tests for rare variants

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

Gene	P-value	Odds Ratio
COL7A1	0.028	4.55
CDX1	0.016	3.86
VEGFA	0.001	6.65
DSE	0.037	3.45

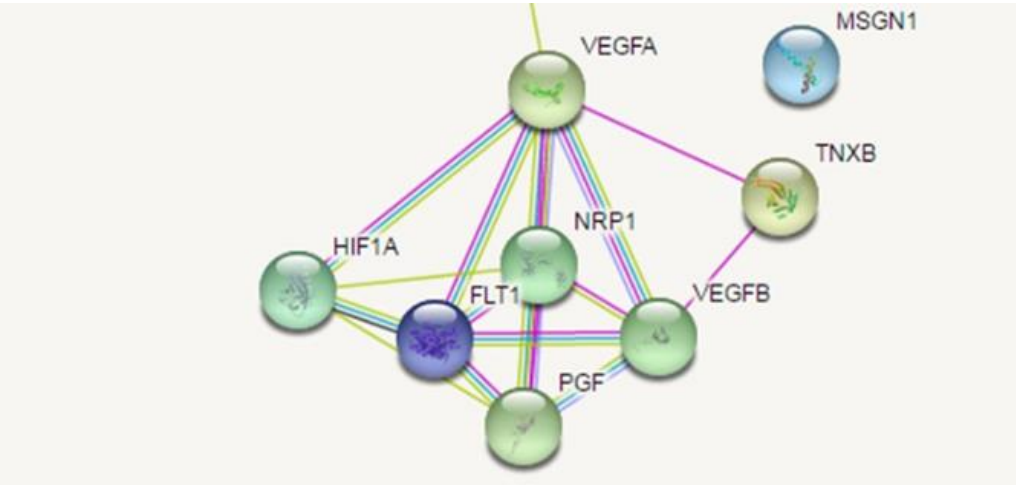
What do these genes have to do with Chiari?

- 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



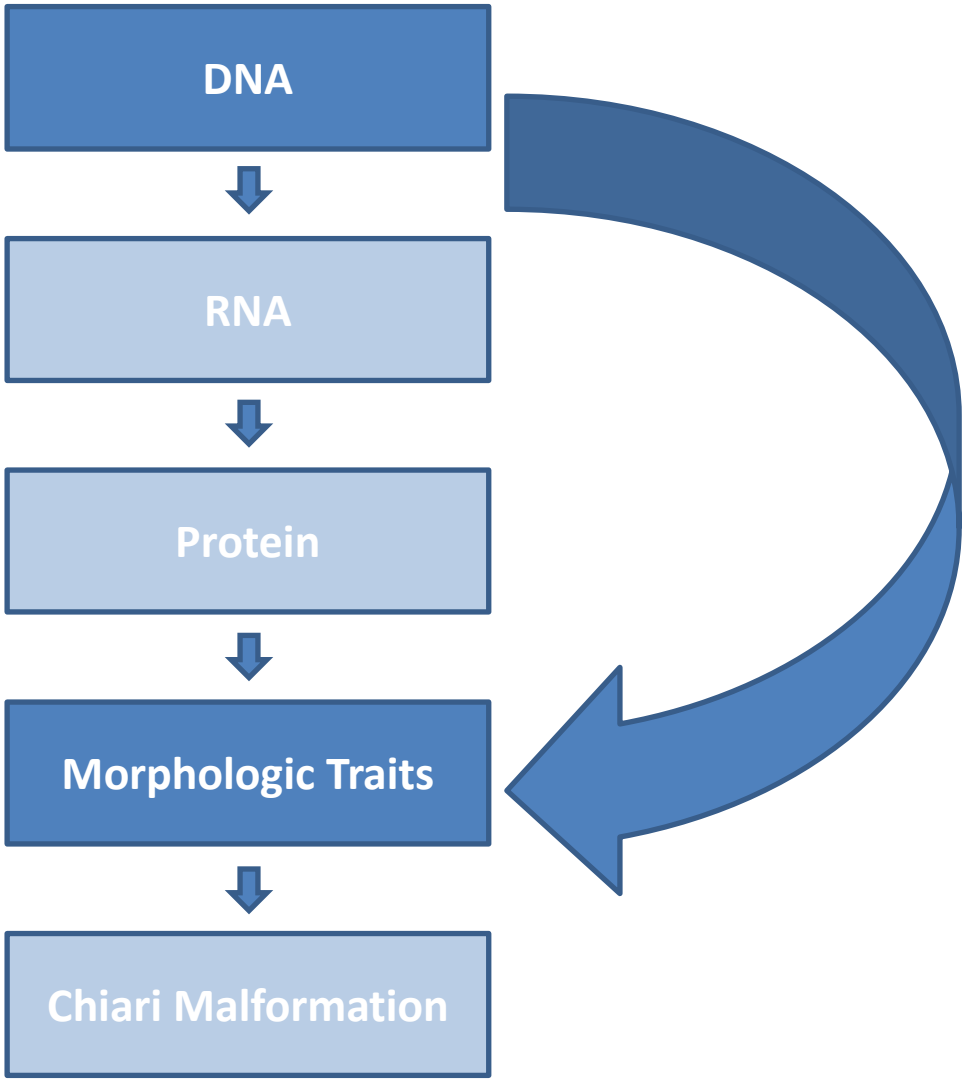
NextGen Sequencing: Summary

- 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!

Future Directions



- 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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NIH National Institute of Neurological Disorders and Stroke