Genetics and Genomics of Craniosynostosis Syndromes

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Craniosynostosis

• Premature fusion of one or several sutures of the skull

• Prevalence: 1 in 2,100 to 1 in 3,000 at birth
Anatomy of Cranial Bones and Bone Growth in Calvaria

Figure Legend: FB: frontal bone; IFS: interfrontal suture, PB: parietal bone, SS: Sagittal suture, LS: lambdoid suture, SOB: supraorbital bone

Abnormal Head Shapes

Unicoronal Synostosis

Lambdoid Synostosis
Positional Deformity vs. Unilateral Lambdoid Synostosis
Severe Proptosis in Patient with Pfeiffer Syndrome

OR: lateral orbital rim; C: cornea
Isolated Nonsyndromic Craniosynostosis

Sagittal synostosis: 50-60%
Coronal synostosis: 20-30%
Metopic and lambdoidal synostosis less common

Etiology: mostly sporadic, but familial instances are known
Isolated Scaphocephaly
Isolated Trigonecephaly
Left-sided Unicoronal Synostosis
Isolated Brachycephaly
Unilateral Lambdoid Synostosis
Syndromic Craniosynostosis

Craniosynostosis with associated anomalies (mostly limb defects)
Over 100 syndromes with craniosynostosis
Sporadic Pfeiffer Syndrome
Mutations in *FGFR1* and *FGFR2* cause familial and sporadic Pfeiffer syndrome

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Identical mutations in three different fibroblast growth factor receptor genes in autosomal dominant craniosynostosis syndromes

Gary A. Bellus¹,², Karin Gaudenz³,
Elaine H. Zackai³, Lorne A. Clarke⁴, Jinny Szabo¹,
Clair A. Francomano¹,² & Maximilian Muenke³

allowing rapid screening of DNA from all available family members. The disease phenotype cosegregated with the mutant allele in both families (Fig. 2b). In contrast, the mutation was not detected in over 120 normal chromosomes. We next screened DNA from 65 unrelated individuals with craniosynostosis with or without limb involvement and were able to identify an additional eight samples with this mutation. On clinical examination, five families segregated non-syndromic craniosynostosis, the remaining three had some clinical findings that were con-

nature genetics volume 14 october 1996

A Unique Point Mutation in the Fibroblast Growth Factor Receptor 3 Gene (FGFR3) Defines a New Craniosynostosis Syndrome

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J. B. Mulliken,⁴ A. E. Guttmacher,⁵ R. S. Wilroy,⁶ L. A. Clarke,⁷ G. Hollway,⁸ L. C. Adès,⁸
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Muenke Syndrome

- Defined by FGFR3 mutation: p.Pro250Arg
- Most common craniosynostosis syndrome
- Muenke syndrome comprises 25% of molecularly defined craniosynostosis
- An estimated 8% of craniosynostosis patients have Muenke syndrome
- Incidence: 1 in 30,000
 Syndromic Craniosynostosis

Apert Syndrome  
Pfeiffer Syndrome  
Crouzon Syndrome  
Muenke Syndrome  
Saethre-Chotzen Syndrome  
Craniofrontonasal Syndrome  

FRFR2  
FGFR1 & FGFR2  
FGFR2  
FGFR3  
TWIST1  
EFNB1
Apert Syndrome
Apert Syndrome
Pfeiffer Syndrome
FGFR mutations in Pfeiffer syndrome
Crouzon Syndrome
FGFR2 Mutations in Crouzon Syndrome
Saethre-Chotzen Syndrome
TWIST1 Mutations in Saethre-Chotzen Syndrome

TWIST1
14 mutations in 16 subjects
Hand and Foot Findings in Patients with Craniosynostosis Syndromes

- Pfeiffer Syndrome
- Saethre-Chotzen Syndrome
- Muenke Syndrome
Craniofrontonasal Syndrome (CFNS)

Craniofacial findings:
- Coronal synostosis (brachycephaly/plagiocephaly)
- Facial asymmetry (with unicoronal synostosis)
- Hypertelorism with down-slanting palpebral fissures & broad nasal root
- Grooved nasal tip
- Cleft lip+/- palate (occasionally)

Other findings:
- Various skeletal anomalies

Inheritance: X-Linked
Paternal grandmother of proband

Father of proband

Proband
EFNB1 Mutations in Craniofrontonasal Syndrome

EFNB1
6 mutations in 6 subjects

EPH    TM    PDZ
Johnson and Wilkie, 2011

**FGFR2**
- 16 mutations in 27 subjects

**FGFR3**
- 2 mutations in 21 subjects (+additional 5 without crs)

**TWIST1**
- 14 mutations in 16 subjects

**EFNB1**
- 6 mutations in 6 subjects

**Key to phenotypes**
- Apert
- Saethre-Chotzen
- Crouzon
- Craniofrontonasal
- Pfeiffer
- Hypochondroplasia
- Muenke
- Syndromic/nonsyndromic crs
- Crs not present

**Key to mutation types**
- Missense
- Nonsense
- Splice site
- Small deletion
- Large deletion

1x multiple
3x bicornoral
7x unicoronal
Genetic Workup of Craniosynostosis

Positive family history
+/− extracranial malformations
+/− profound developmental delay

Clinical Genetics

Involved cranial sutures/resulting head shape

Unclassified coronal Cs/Brachycephaly, anterior plagiocephaly

Cloverleaf skull
Pansynostosis

Brachycephaly, Plagiocephaly

Sagittal Cs/Scaphocephaly

metopic Cs/Trigonocephaly

Isolated, Muenke, Crouzon, SCS, mild Pfeiffer, JWS

Severe Crouzon or Pfeiffer

Apert

CFNS

Early Crouzon, Carpenter

Rubinstein-Taybi, Optiz C, others

FGFR3 exon 7(IIIa)+10, FGFR2 exon 8+10(IIIa+c)

FGFR1 exon 7(IIIa)
TWIST exon 1

FGFR2 exon 3, 5, 11, 14-17

Craniofacial MLPA


EFNB1 incl. MLPA

e.g. RAB23, FGFR2

Array CGH +/- karyotyping

FGFR2

Hehr, 2011
Muenke Syndrome
1. Ligand-dependent gain-of-function mutations act by increasing ligand-binding affinity and by overriding ligand-binding specificity of affected receptors.

2. Exclusive paternal origin, if de novo.
Fibroblast Growth Factor Receptors

1. Ligand-dependent gain-of-function mutations act by increasing ligand-binding affinity and by overriding ligand-binding specificity of affected receptors

2. Exclusive paternal origin, if de novo
Muenke Syndrome

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• Incidence: 1 in 30,000
Muenke Syndrome
### Phenotypic Features

<table>
<thead>
<tr>
<th>Finding</th>
<th>Estimated prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniosynostosis</td>
<td>83</td>
</tr>
<tr>
<td>Bilateral coronal craniosynostosis</td>
<td>55</td>
</tr>
<tr>
<td>Unilateral coronal craniosynostosis</td>
<td>26</td>
</tr>
<tr>
<td>Other craniosynostosis type</td>
<td>5</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>3</td>
</tr>
<tr>
<td>Hearing loss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>35</td>
</tr>
<tr>
<td>Hand anomalies</td>
<td>65</td>
</tr>
<tr>
<td>Foot anomalies</td>
<td>55</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hearing loss estimated to be variable.
Muenke Syndrome (FGFR3-Related Craniosynostosis): Expansion of the Phenotype and Review of the Literature


Muenke syndrome is an autosomal dominant disorder characterized by coronal suture craniosynostosis, hearing loss, developmental delay, carpal and tarsal fusions, and the presence of the Pro250Arg mutation in the FGFR3 gene. Reduced penetrance and variable expressivity contribute to the wide spectrum of clinical findings in Muenke syndrome. To better define the clinical features of this syndrome, we initiated a study of the natural history of Muenke syndrome. To date, we have conducted a standardized evaluation of nine patients with a confirmed Pro250Arg mutation in FGFR3. We reviewed audiograms from an additional 13 patients with Muenke syndrome. A majority of the patients (95%) demonstrated a mild-to-moderate, low frequency sensorineural hearing loss. This pattern of hearing loss was not previously recognized as characteristic of Muenke syndrome. We also report on feeding and swallowing difficulties in children with Muenke syndrome. Combining 312 reported cases of Muenke syndrome with data from the nine NIH patients, we found that females with the Pro250Arg mutation were significantly more likely to be reported with craniosynostosis than males \( (P < 0.01) \). Based on our findings, we propose that the clinical management should include audiometric and developmental assessment in addition to standard clinical care and appropriate genetic counseling. Published 2007 Wiley-Liss, Inc.

Key words: craniosynostosis; Muenke syndrome; fibroblast growth factor receptor 3; coronal suture synostosis; hearing loss; developmental delay; speech delay
Muenke Syndrome Projects in Progress

1. Behavioral phenotype characterization


3. Gene modifiers and phenotypic variation
Cognitive Function, Development, and Hearing in Patients with Muenke Syndrome (FGFR3-Related Craniosynostosis)

Muenke Syndrome, which results from an FGFR3 mutation resulting in p. Pro250Arg, is the most common genetic cause of craniosynostosis, with a prevalence of 1 in 30,000 births. Craniosynostosis is the premature fusion of one or more of the skull sutures. Individuals with Muenke syndrome show a wide variety of features, including premature fusion of one or both coronal sutures (sutures found on the side of the skull), hearing loss, developmental delay, intellectual disabilities, macrocephaly (large head size) and radiographic changes. There are also individuals with Muenke syndrome who have no symptoms or signs of this syndrome.

We have ongoing studies at the National Institutes of Health (NIH) that focus on various aspects of Muenke syndrome, and we hope to improve our understanding of hearing, cognitive function, and development in people with Muenke syndrome.

We are currently conducting research on the relationship between development, cognitive function and hearing in individuals with Muenke syndrome. The goal of this study is to better understand the development of the central nervous system as well as to understand the causes of developmental delay and intellectual disabilities that can occur in some individuals with Muenke Syndrome. This study will also help us to learn much about the long term outcomes and functioning of adults with Muenke syndrome. In addition, we also hope to be able to outline factors that may contribute to and predict mental prognosis in individuals with Muenke syndrome. Please note, you do not need to have developmental delay, intellectual disabilities, or hearing loss in order to participate in our study.
Behavioral Phenotype
Craniosynostosis
Hearing loss by report
FGFR3 p.P250R mutation positive
Muenke Syndrome

“Behavioral” Phenotype

Craniosynostosis

Hearing loss (by report)

FGFR3 p.P250R mutation positive

Behavioral Phenotype (by report)
Validated Behavioral Screening Tools

- Vineland Adaptive Behavior Scales-II: Structured Interview form or Parent response
- ABAS-II
- Social Responsiveness Scale (SRS): parent response
- Social Communication Questionnaire (SCQ): parent response
Non-syndromic FGFR3 p.P250R
Deafness
Deafness as Single FGFR3 p.P250R Manifestation

Hollway et al. THE LANCET 1998
Does the FGFR3 p.Pro250Arg Contribute to Non-Syndromic Deafness?

FGFR testing in DNA samples from deaf or hearing impaired individuals in collaboration with

- Kathleen Arnos, Gallaudet University
- Arti Pandya, Virginia Commonwealth University
- Richard Smith, University of Iowa
- Heidi Rehm, Harvard
Gene Modifiers and Phenotypic Variation
## TABLE 1. Clinical Manifestation of Muenke Syndrome

<table>
<thead>
<tr>
<th>Finding</th>
<th>Previously recognized findings (% of affected patients)</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicoronal synostosis</td>
<td>60</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Unicoronal synostosis</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midface hypoplasia</td>
<td>50</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Downslanting palpebral fissures</td>
<td>56</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ocular hypertelorism</td>
<td>78</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ptosis</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing loss</td>
<td>33</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Developmental delay</td>
<td>35</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>40</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clinodactyly</td>
<td>35</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cerebral white-gray matter anomalies</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Gyri</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal hippocampus</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Additional findings in current report

<table>
<thead>
<tr>
<th>Finding</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porencephaly</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Hydrocephaly</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Agenesis of the corpus callosum</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Tracheo-esophageal fistula</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>ADHD</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>PDD</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder; PDD, pervasive developmental disorder.

*Previously recognized findings based on Graham et al. [1998] and Grosso et al. [2003]. Percentage of patients with finding.
Pooling/bootstrap-based GWAS (pbGWAS) identifies new loci modifying the age of onset in PSEN1 p.Glu280Ala Alzheimer’s disease

The literature on GWAS (genome-wide association studies) data suggests that very large sample sizes (for example, 50,000 cases and 50,000 controls) may be required to detect significant associations of genomic regions for complex disorders such as Alzheimer’s disease (AD). Because of the challenges of obtaining such large cohorts, we describe here a novel sequential strategy that combines pooling of DNA and bootstrapping (pbGWAS) in order to significantly increase the statistical power and exponentially reduce expenses. We applied this method to a very homogeneous sample of patients belonging to a unique and clinically well-characterized multigenerational pedigree with one of the most severe forms of early onset AD, carrying the PSEN1 p.Glu280Ala mutation (often referred to as E280A mutation), which originated as a consequence of a founder effect. In this cohort, we identified novel loci genome-wide significantly associated as modifiers of the age of onset of AD (CD44, rs127116, \( P = 1.29 \times 10^{-12} \); NPHP1, rs10173717, \( P = 1.74 \times 10^{-12} \); CADPS2, rs3757536, \( P = 1.54 \times 10^{-10} \); GREM2, rs12129547, \( P = 1.69 \times 10^{-13} \), among others) as well as other loci known to be associated with AD. Regions identified by pbGWAS were confirmed by subsequent individual genotyping. The pbGWAS methodology and the genes it targeted could provide important insights in determining the genetic causes of AD and other complex conditions.

Molecular Psychiatry (2013) 18, 568–575; doi:10.1038/mp.2012.81; published online 19 June 2012

Keywords: Alzheimer’s disease; bootstrap; DNA pooling; GWAS; modifiers; PSEN1
How can we exploit classical genetics or genomics to identify modifiers of human phenotypes for Muenke syndrome?

Classical genetics:

1) Selective breeding of the murine *Fgfr3* mutant transgenic lines into new strain backgrounds to look for penetrance or phenotypic variability (labor intensive)
2) Sequence/Genotype known pathway components or targets of FGFR3 signaling (rational candidate approach)
3) Derive new candidate genes by over-expressing the mutant FGFR3 vs. the WT version in various animal models (presumed dominant mutation with novel signaling properties)
4) Then interrogate such genes for variants that correlate with phenotypes
Muenke Syndrome: A Single Mutation Several Phenotypes

Modifier Genes

Environment

Comprehensive Inventory Using Next Generation Sequencing: Targeted \textit{FGFR3} Deep Sequencing, WES, or WGS.

- Rare Variants (Not present in databases)
- De novo mutations (Parent Analysis)
- Compound Heterozygotes or Homozygotes
- Epigenomics and Functional Analysis (PolyPhen, SIFT)

Craniosynostosis

Hearing loss by report

\textit{FGFR3} p.P250R mutation positive

+ + - + - + - + +
Identifying modifier genes for a specific trait will depend on

1) Availability of families segregating the FGFR3 Pro250Arg mutation
2) Number of mutation carriers in each family
3) Number of mutation carriers with and without the trait (e.g. craniosynostosis, hearing, cognition, behavior)
Thank you

**NHGRI**
Amaka Agochukwu, now Univ. of Kentucky
Paul Kruszka
Don Hadley
Maria Guillen
Ben Solomon, now INOVA
Erich Roessler
Suzanne Hart
Tyler Carney
Colin Yarnell

**Collaborators**
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Carmen Brewer, NIDCD, NIH Clinical Center
Kathy Arnos, Gallaudet University
Arti Pandya, Virginia Commonwealth University
Heidi Rehm, Harvard
Richard Smith, University of Iowa
Hartmut Collmann, University of Würzburg
Mauricio Arcos-Burgos, Australian National University
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Molecular Genetics, Principles of Diagnosis and Treatment

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