FISH and the Face
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Principle:
If a patient has two or more minor malformations, an occult major malformation (or syndrome) is likely

Major vs Minor Malformation
- Major malformation
  - Arrest in morphogenesis
  - Affects function
  - Affects societal acceptance
- Minor malformation
  - Arrest in morphogenesis
  - No effect on function or societal acceptance

Major Malformation

Minor Malformation

Minor Anomalies

<table>
<thead>
<tr>
<th>Number of Minor Anomalies</th>
<th>No Major Anomaly</th>
<th>One Major Anomaly</th>
<th>Multiple Major Anomalies</th>
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<tbody>
<tr>
<td>Number</td>
<td>Percentage</td>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
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Marden et al., 1964 (n=8412)
Making a Diagnosis

- Clinical vs laboratory diagnosis
- Clinical diagnosis
  - No biomarker
  - “It is because I say it is”
  - Example: fetal alcohol syndrome
- Laboratory diagnosis
  - Associated with biomarker

. History:
  Prenatal alcohol exposure
  Examination:
  - Small head
  - Small eyes
  - Smooth philtrum
  - Thin vermilion
  . Diagnosis?

A Newborn with an Asymmetric Mouth

- Your diagnosis?
- Next steps?

Genetic Biomarkers

- Establish/exclude a diagnosis
- Aid in prognostication
- Aid in recurrence risk counseling
- Identify at-risk relatives
- Provide opportunities for ongoing care

Diagnosis?
Next steps?

Craniofacial Genetic Diagnosis

- Clinical
  - Laboratory
    - Chromosomal analysis
    - Fluorescence in situ hybridization (‘FISH’)
    - Direct DNA analysis
    - Chromosomal microarray analysis (CMA)
Approach to Clinical Diagnosis

- We assess
  - Facial proportion such as...
    - Upper face (e.g., prominent forehead)
    - Middle face (e.g., midface hypoplasia)
    - Lip/philtrum complex
    - Lower face (e.g., micrognathia)
  - Facial or cranial asymmetry
    - Facial asymmetry
    - Craniosenosis

Craniofacial Genetic Diagnosis

- Clinical
- Laboratory
  - Chromosomal analysis
  - Fluorescence in situ hybridization (“FISH”)
  - Direct DNA analysis
  - Chromosomal microarray analysis (CMA)

Diagnosis?

- Craniosynostosis
- Mitten syndactyly
- Variable clefting
- Mutation in FGFR2
- Allelic with Crouzon syndrome
- Paternal age effect
Crouzon Syndrome

- Autosomal dominant
- Allelic with Apert syndrome
- FGFR2 mutations

Craniofacial Genetic Diagnosis

- Clinical
- Laboratory
  - Chromosomal analysis
  - Direct DNA analysis
  - Fluorescence in situ hybridization ("FISH")
  - Chromosomal microarray analysis (CMA)

Toddler with developmental delays...

What do you see?

Diagnosis?

Astley’s Likert lip-Philtrum Guide

From Astley et al, 1996

FAS “Look alikes”

- Williams syndrome (ELN 7q23 deletion)
- VCF/DGS syndrome (22q11.1 deletion)
- Anticonvulsant embryopathy
- Maternal PKU embryopathy
- Juvenile bipolar illness
- Dubowitz syndrome
The Phenotype

- Growth deficiency, mild
- Microcephaly, mild
- "Cocktail party" personality
- Wide mouth, full lips
- Stenotic vascular lesions
  - PS, SVAS, RAS
- FISH - deletion in ELN region on 7q11.23

Fluorescence in situ hybridization (“FISH”)

- Developed in the 1990s
- Useful for clinically identifiable microdeletion syndromes that cannot be detected using standard cytogenetic methods
  - Deletion 22q11.2 (velocardiofacial syndrome)
  - Williams syndrome
- Also for rapid detection of common aneuploidy in amniotic fluid samples

Fluorescence in situ Hybridization

- Six-year-old
- Developmental delays
- Smooth philtrum
- Small alae nasi
- Anomalous auricle
- Diagnosis?
Boy with Developmental Delays and Hoarse Voice
- Velopharyngeal insufficiency (VPI)
- Hypermusal speech
- “Narrow” palpebral fissures
- Long nose/thin alae nasi

The Phenotype
- Growth –
  - Feeding difficulties/VPI
  - Short stature, postnatal ~60%
- Performance
  - Normal to mild learning disabilities ~60%
  - IQ 70 – 90+
  - Thought disorder/psychosis 10%
- Craniofacial
  - Narrow palpebral fissures
  - Midface hypoplasia
  - Smooth philtrum (Astley 4 – 5/5)
  - Thin vermilion (Astley 4 – 5/5)
  - Hypoplasia of submucosal palatal musculature
  - Robin sequence
  - Anomalous auricles

Review of Cases at WCHOB
- n = 25
- male = 11/25 (44%)
- Common presentations
  - Murmur – 24%
  - “Dysmorphic” – 28%
  - Velopharyngeal insufficiency – 16%
  - Developmental delay – 12%
  - Psychosis – 4%

The Phenotype
- Limbs
  - Long gracile fingers
  - Mild cutaneous syndactyly
- Other
  - Cardiac defects, VSD, conotruncal ~85%
  - Hypocalcemia
  - Thymic hypoplasia/immunodeficiency

Craniofacial Genetic Diagnosis
- Clinical
- Laboratory
  - Chromosomal analysis
  - Fluorescence in situ hybridization (“FISH”)
  - Direct DNA analysis
  - Chromosomal microarray analysis (CMA)
Case

- Three-month of female with VSD, poor weight gain, small head small head, Third born child to a 23-year-old healthy woman and her 23-year-old healthy nonconsanguineous husband
- Family history was remarkable for a maternal uncle with a VSD; several maternal relatives also had learning disabilities

Family History

- Mother: 23-years-old; healthy
- Father: 23-years-old; healthy
- No consanguinity
- Maternal uncle has VSD; several maternal relatives also had learning disabilities

Prenatal and Birth History

- 40 weeks' gestation
- Prenatal exposure to varicella at 6 months of pregnancy; mother did not develop chicken pox
- Vaginal/vertex delivery; birth weight was 5#5oz
- NICU admission for transient respiratory distress and hypoglycemia; discharged at 4 days of age

Newborn Examination

- Weight at (term) birth: 2.6 kg
- Length – 50 cm
- Clinical findings
  - Growth deficiency, mild
  - Hypotonia, mild
  - Large (3 cm x 3 cm) anterior fontanelle

Postnatal History: Medical

- Cardiology: VSD diagnosed at age 4 months; required surgical closure
- Neurology: parents suspected seizures beginning at age 4 months; negative work-up
- Ophthalmology: pseudostrabismus; otherwise normal
- Audiology: normal hearing

Diagnostic Studies

- EEG: normal
- Echocardiogram: VSD and PDA
- MRI brain: normal
- Routine karyotype:
Developmental History
- Motor and speech delays, severe
  - Hypotonia during infancy
  - Walked independently at age 34 months
  - No true speech; 2-3 consistent signs

Physical Examination
- Age: 3 years 6 months
- Growth:
  - Height: 88 cm (<5th centile; 50th centile for 27 months)
  - Weight: 12 kg (3rd centile; 25th-50th centile for 27 months)
  - OFC: 45 cm (<3rd centile; 3rd centile for 27 months)

Craniofacial
- Straight eyebrows
- Depressed nasal bridge
- Short columella

Extremities
- Short fifth fingers

Assessment
- 3½-year-old with unrecognized pattern of malformation (i.e., no diagnosis assigned)
  - Growth deficiency
  - Hypotonia, global delays, absent speech
  - Small head
- Diagnostic considerations: submicroscopic chromosomal abnormality
Diagnostic Studies

- Chromosomal microarray analysis (CMA):
  - Submicroscopic deletion at 1p36

Chromosomal Microarray Analysis

- Examines genetic “hot-spots” for chromosomal deletions and duplications throughout the genome
- Detects copy number variations at resolution of 1Mb or less
- Detection rate 10-20% for individuals with normal karyotype, unexplained developmental delay/MR +/- dysmorphic features

Chromosomal Microarray Analysis

Deletion 1p36: A Recognizable Pattern of Malformation

- Most common terminal chromosomal deletion, occurring in 1/5000 live births
- Accounts for ~1% of cases of unexplained mental retardation
- 2-3% of the general population has mental retardation, 50% of which have no identifiable etiology (genetic or environmental)

Deletion 1p36: The Phenotype

- Growth: postnatal growth deficiency (85%)
- Development:
  - Feeding difficulties: oropharyngeal dysphagia with failure to thrive
  - Hypotonia/developmental delays (95%)
  - Seizures (45%)
  - Severe to profound mental retardation with absent or very little speech

Deletion 1p36: The Phenotype

- Craniofacial:
  - Microcephaly (95%)
  - Large anterior fontanelle
  - Decreased AP dimension of the head
  - Straight eyebrows
  - Deeply set eyes
  - Depressed nasal bridge
  - Pointed chin
Deletion 1p36: The Phenotype

- Cardiac defects (70%)
- Hearing loss (28%)

WCHOB Experience: arrCGH

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<th>Diagnosis</th>
<th>n</th>
<th>%</th>
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<td>Intellectual developmental delay</td>
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<tr>
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<tr>
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Microarray Analysis

- Higher resolution than any previously available cytogenetic technique
- Potential diagnosis of previously unrecognized patterns of malformation

Microarray Analysis - Considerations

- Pathologic change or parental polymorphism?
  - Some *benign copy number* changes may be familial polymorphisms
- Prognosis may be unknown for *de novo* rearrangements
- Need for pre-authorization (carriers and NYSDOH)
Microarray Analysis - Indications

- Patients with unexplained cause of birth defects mild or more severe mental retardation
  - Growth deficiency
  - Mental deficiency
  - Microcephaly
  - Craniofacial anomalies
- “Gestalt”, “looks chromosomal”