Chromosome 22
The Commonly Deleted Region

DiGeorge Critical Region
The q11 Deletion

◆ Tissues forming the conotruncal region, thymus, and parathyroid have common embryonic origin at about 4th -5th week gestation

◆ The q11 region of chromosome 22 “houses” the genes involved in normal development of:
  - the thymus
  - the parathyroid gland
  - the conotruncus
  - the palate
The q11 Deletion (cont’d)

- A deletion of this region and its corresponding genes ➔ abnormal development and physiology of those structures which derive from the 3rd and 4th pharyngeal pouches

- This microdeletion may vary slightly between individuals and thus explains the range and severity of problems from one person to the next
Significance of 22q11 Deletion

- 22q11.2 deletion = underlying cause of multiple medical problems found in patients with DiGeorge syndrome, Velocardiofacial Syndrome, and Conotruncal Anomaly Face Syndrome.

- These are very similar syndromes if not one and the same.
A BIT OF AN HISTORICAL PERSPECTIVE

Which you already know
DiGeorge Syndrome

- In 1965 Dr. DiGeorge first described this “collection of findings” or syndrome
  - Absent or hypoplastic parathyroid leading to calcium abnormalities
  - Absent or hypoplastic thymus resulting in immune deficiencies
  - Conotruncal heart defects - IAA, Truncus arteriosus, TOF, etc.
Conotruncal Anomaly Face Syndrome

- Described by Dr. Kinouchi in 1976
- Conotruncal heart defects
- Characteristic facial features
  - protruding ears, wide set eyes
Velocardiofacial Syndrome

- Described by Dr. Shprintzen in 1978
- Velopharyngeal (soft palate & pharynx) anomalies
- Heart defects
  - TOF, VSD, Right aortic arch
- Characteristic facial features
  - prominent nose, jaw abnormalities
- Learning disabilities
What We Have Learned

1981
- Patients with DiGeorge missing small piece on long arm of chromosome 22

1990
- FISH probe identifies submicroscopic deletion of chromosome 22q11 in majority of patients with DiGeorge (but not all)

1992
- VCFS and DiGeorge are same diagnosis
PERHAPS ONE OF THE MOST IMPORTANT CONCEPTS...
It is a multi-system disorder with many common serious medical problems:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune/Autoimmune disease</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>76%</td>
<td>40% (serious)</td>
</tr>
<tr>
<td>Palatal anomalies</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>49%</td>
<td>65%</td>
</tr>
<tr>
<td>Renal abnormalities</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>Feeding and swallowing problems</td>
<td>35%</td>
<td></td>
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<tr>
<td>Hypothyroidism</td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>Intellectual deficits</td>
<td>&gt; 95%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Psychiatric illness</td>
<td>25%</td>
<td>60%</td>
</tr>
</tbody>
</table>

** All figures may be influenced by ascertainment affects

McDonald-McGinn, 2001; Bassett, 2005
THE HEART
22q11 Deletion

Cardiac Features

◆ Some form of CHD found in 74-85% of affected individuals

◆ Most (but not all) are conotruncal defects
  – IAA = 50% have 22q11
  – Truncus = 57% have 22q11
  – VSD = 25% have 22q11
  – TOF and its variants = 15% have 22q11

◆ Least common in DORV, TGA
How Does This Compare?

• In one series of patients studied…
  – 9 out of 69,129 births had 22q11 deletion
  – 6 of these had significant heart disease
  – 53 out of 69,129 births had trisomy 21
  – 15 of these had significant heart disease

• 22q11 deletion 2nd most common chromosomal cause of significant heart disease
  – (Goodship et. al, 1998)
22q- deletion syndrome and CHD

- Conotruncal abnormalities +
  - TOF
  - Truncus arteriosus
  - TOF with absent pulmonary valve
  - IAA
  - Truncus with IAA
  - Others
Is early diagnosis beneficial?

This may be a little controversial.
A tale of two patients, both with 22q11.2DS and IAA Type B
Tale of two patients – Patient A

- Discharged Day 2 from hospital w/o Dx
- Presented in ER Day 9 in extremis
  - Complicated resuscitation
  - Suffered a stroke
- Transferred to CHW - diagnosed with IAA-B and 22q11.2DS
- Multiple additional surgeries
- Approximately $750,000 in increased hospital charges over first year of life

...and living with a stroke

THE MAJOR CONCERNS REGARDING THE NEUROLOGIC OUTCOME IS CRITICAL!!!
Tale of two patients – Patient B

- Diagnosed prenatally by ECHO
- Started on PGE
- Surgery - Complete repair
- Discharged
Benefits of diagnosis before duct closure for IAA

N=19 IAA patients at CHW

Non Parametric Mann-Whitney Test $p<0.04$
Total inpatient cost related to cardiac issues in the first year of life: Early vs. late diagnosis in IAA

- No shock  n=10  $194,864
- Shock  n=9  $542,987

Average difference in charges  $359,764

Suggests a significant (p<0.04) cost savings within one year of newborn screening (at $6/test) with detection of one patient.

CRITICALLY, THE AIM IS TO AVOID POOR NEURO OUTCOME, IF AT ALL POSSIBLE
WHY?
Early diagnosis of 22q11.2DS can dramatically decrease morbidity and likely mortality

- 40-75% of 22q11.2DS have serious CHD
- Most duct dependent CHD need surgery in the first few weeks of life
- Increased morbidity and mortality with late diagnosis
- Early diagnosis of cardiac abnormalities markedly reduces overall costs
And although we are advocates for neonatal pulse oximetry

Interrupted aortic arch will not likely be discovered by neonatal pulse oximetry screening

AND

Fetal ultrasound is less uniformly performed and can also miss IAA
Newborn screening for 22q11.2DS

- Can it be done accurately? Logistically? Cheaply? Rapid turnaround?

- A suitable screening test exists

- Is this at all feasible and ready for prime time?
Multiplexed quantitative real-time PCR to detect 22q11.2 deletion in patients with congenital heart disease

Aoy Tomita-Mitchell,1 Donna K. Mahnke,1 Joshua M. Larson,1 Sujana Ghanta,2 Ying Feng,1 Pippa M. Simpson,3,4 Ulrich Broeckel,4 Kelly Duffy,5 James S. Tweddell,1 William J. Grossman,6 John M. Routes,4,7 and Michael E. Mitchell1

1Division of Cardiovascular Surgery, Department of Surgery, 3Division of Quantitative Health Sciences, 4Department of Pediatrics, 5Department of Dermatology, 7Allergy, Asthma, and Clinical Immunology Program, Medical College of Wisconsin, Milwaukee, Wisconsin; 2Department of Computer Science, University of Louisville, Louisville, Kentucky; and 6Baxter Healthcare Corporation, Deerfield, Illinois

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Tomita-Mitchell A, Mahnke DK, Larson JM, Ghanta S, Feng Y, Simpson PM, Broeckel U, Duffy K, Tweddell JS, Grossman WJ, Routes JM, Mitchell ME. Multiplexed quantitative real-time PCR to detect 22q11.2 deletion in patients with congenital heart disease. Physiol Genomics 42A: 52–60, 2010. First published June 15, 2010; doi:10.1152/physiolgenomics.00073.2010.—22q11.2 Deletion syndrome (22q11.2 DS) [DiGeorge syndrome type 1 (DGS1)] occurs in ~1:3,000 live births; 75% of children with DGS1 have severe congenital heart disease requiring early intervention. The gold standard for detection of DGS1 is fluorescence in situ hybridization (FISH) with a probe at the TUPLE1 gene. However, FISH is costly and is typically ordered in conjunction with a karyotype analysis that takes several days. Therefore, FISH is underutilized and the diagnosis of 22q11.2 DS is frequently delayed, often resulting in profound clinical consequences. Our goal was to determine whether multiplexed, quantitative real-time PCR (MQPCR) could be used to detect the haploinsufficiency characteristic of 22q11.2 DS. A retrospective blinded study was performed on 382 subjects who had undergone congenital heart surgery. MQPCR was performed with a probe localized to the TBX1 gene on human chromosome 22, a gene typically differences between related individuals with identical 22q11.2 microdeletions (2, 9, 11, 12, 23, 27). Clinical abnormalities are diverse and include psychosocial, cognitive, developmental delay, psychiatric illnesses, palatal abnormalities, parathyroid insufficiency, growth retardation, immune defects, congenital heart defects (CHDs), renal anomalies, and abnormal craniofacial findings (23, 24). The early diagnosis of 22q11.2 DS is critically important to effectively treat this disorder. However, despite the obvious phenotypic abnormalities in many patients with 22q11.2 DS, the average age of diagnosis for a person with this syndrome is 6–7 yr (27). Because of its highly variable phenotype, 22q11.2 DS has also been known by a variety of other names (24, 34).

Currently, the definitive diagnosis of a patient with suspected 22q11.2 DS relies on the fluorescent in situ hybridization (FISH) cytogenetic test using a probe localized to the TUPLE1 gene. This probe lies within the ~3-Mb typical deleted region (TDR) found on chromosome 22q11.2. Unfor-
MP-QRT TBX1 assay in 382 Subjects with CHD

Relative Gene Copy Number - CNV Assay

Rapid, low cost, amenable to high-throughput
Confirmed by Affymetrix 6.0 analysis

- **Sensitivity 100% (21/21)**
- **Specificity 100% (361/361)**
DNA from 80 NBS cards and 2 with 22q11.2DSs

Individuals with 22q11.2DS can be identified
Use of MP-QRT PCR to detect 22q11.2DS

MP-QRT PCR for \textit{TBX1} haploinsufficiency

- Sensitive and specific for 22q11.2DS
- Amenable to high-throughput screening
- Inexpensive (estimated cost $6 per assay)
- Uses technology in use in state labs (newborn screening assay for SCID is MP-QRT)
- The tricky question: get the result back quickly?
What about the other systems that are affected by 22q11.2ds?

You will hear more about this from others today
Palate Problems

- Occur in 30-70%
  - Velopharyngomaline incompetence (VPI) (27%)
  - Cleft palate (16%)

- Palatal function: a major role in speech development

- Studies: majority of patients will manifest some degree of VPI or hypernasal speech
Palate Problems

- Velopharyngeal valve: the portion of pharynx that is open at rest and closes during speech, swallowing, crying
- Children with VPI: unable to achieve closure with speech and air escapes through the nose producing hypernasal resonance
- May see nasal regurgitation of formula
Palate Problems

- Diagnosis made through history, speech evaluation, nasoendoscopy, or videofluoroscopy
- May need Plastic Surgery consultation
Endocrine Problems

- Hypocalcemia related to hypoplasia of parathyroids
  - Usually seen in infancy and outgrown
  - May need calcium supplement

- Growth Retardation as some affected persons may have small pituitary
  - May respond to growth hormone therapy
Immune Deficiency

- No thymus or hypoplasia of thymus may impair T-cell production and function

- Recommendations include no live vaccines and irradiated blood

- Deficiency usually outgrown by 1 year
Feeding Disorders

- In addition to nasopharyngeal reflux from VPI, may see dysmotility in the pharyngoesophageal area which is derived from the 3rd and 4th pharyngeal pouches.

- Speech / Feeding consult strongly suggested.

- NG or GT feeds often needed during infancy.
ENT Problems

- Chronic otitis media and chronic sinusitis likely from NP reflux

- Conductive hearing loss related to infections /fluid - audiology evaluations should be considered
Neurodevelopmental Problems

- One study: 40 preschoolers and toddlers with 22q11.2 deletion
  - Mental developmental delay (33%)
  - Motor developmental delay (79%)
  - Language delay (all had delay)
  - Speech delay (all had late onset of speech)
    - (Gerdes et. al, 1999)
Facial Features of Child with 22q11 Deletion

- Wide set eyes
- Protruberant ears
- Hooded upper eyelids
- Preauricular pits or tags
- Narrow ear canals
- Small mouth with thin upper lip
- Prominent nose
HOW SHOULD WE SCREEN FOR 22Q11.2 DELETION?

FISH in suspected cases?
Microarray in suspected cases?
MP-QRT TBX1 PCR screen in all newborns?
Not at all?
Standard Screening for 22q11 Deletion?

- There is controversy about the utility and effectiveness of screening for 22q11 deletion.
- Some support FISH deletion studies in patients with conotruncal heart anomalies.
- Others oppose such screening as it shows no predictive value regarding outcomes.
- Recently some have suggested FISH analysis on amniotic fluid if heart defect is suggested on prenatal ultrasound.
Summary/Overall Considerations in 22q11 Deleted Children

- Once the genetic diagnosis is made consider both the needs of the child and the family

- These children will likely have a collection of problems

- A multidisciplinary, comprehensive evaluation at the time of diagnosis will best meet the needs of these children AND IS CRITICAL
Thank you very much