22q11.2 Deletion/Duplication Syndromes

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This morning I will be discussing:

1. A brief history of the 22q11.2 deletion
2. Laboratory studies for the detection of the deletion
3. Clinical findings including:
   - frequency
   - size of the deletion
   - inheritance patterns/recurrence risks
   - associated features
   - findings in adults
4. Atypical deletions
5. 22q11.2 duplications
6. Suggested evaluations
7. Prenatal clues to the diagnosis
Historically, the 22q11.2 deletion has been identified in the majority of patients with:

- DiGeorge
  - De la Chapelle, 1981
  - Kelley, 1982

- VCFS
  - Driscoll, 1992
  - Scambler, 1992

- CTAF
  - Burn, 1993
And in some patients with:

Opitz G/BBB
- McDonald-McGinn, 1995
- Lacassie, 1997
- Fryburg, 1997

Cayler Cardiofacial
- Giannotti, 1994
A group of specialized veterinarians trying to identify an elephant by each examining separate parts.

Each was accurate in describing his own area of interest.

But none was able to see the big picture.

So too was the case of the 22q11.2 deletion...
For example:

In 1965, Angelo DiGeorge, M.D. first described patients with:

- Hypocalcemia
- Immune Deficiency
- Later – Congenital Heart Disease was added
In 1982, Elaine Zackai, M.D. identified this patient with clinical features of DiGeorge syndrome including:

- Truncus arteriosus
- Hypocalcemia
- Immune deficiency
- Cleft palate
- Jejunal web

Kelley, 1982
In whom chromosome studies revealed:

- only 45 chromosomes
- including a 10;22 unbalanced translocation
- resulting in a deletion in both chromosome 10 and chromosome 22

Kelley, 1982
Concurrently:

Several other patients with DiGeorge syndrome came to attention with both a 22q11.2 deletion and some other chromosomal difference, so…

…the conclusion was drawn that DiGeorge syndrome was due to a chromosome 22q11.2 deletion

De la Chapelle, 1981
Kelley, 1982
Between 1982 and 1992 visible deletions were identified in 25% of patients with DiGeorge syndrome. But the puzzle remained – what about the remaining 75% of patients with no visible deletion?

Scambler, 1991; Driscoll, 1992; Wilson, 1992
This led to the development of FISH probes to detect smaller deletions which were not visible under the microscope.

Often referred to as submicroscopic deletions

Scambler, 1991; Driscoll, 1992
So since 1992 the 22q11.2 deletion has most often been identified using FISH studies as seen here:

Scambler, 1991; Driscoll, 1992
However, recent advances in the laboratory are making identification easier and allowing for the detection of even smaller deletions.

Emanuel and Saitta, 2007
These techniques include:

1. Comparative Genomic Hybridization (CGH)

2. Whole Genome Array/SNP Arrays (WGA)

3. Multiplex Ligation-dependent Probe Amplification (MLPA)
Comparative Genomic Hybridization (CGH)
Multiplex Ligation-dependent Probe Amplification (MLPA)

22q11.2 deletion
In looking at frequency, we know a fair amount about the prevalence of the 22q11.2 deletion in certain populations.
For example:

- It has an estimated prevalence of 1 in 4000 live births
- It is present in 1 of 68 children born with congenital heart disease
- It is the most frequent syndromic cause of palatal defects
It is so common that there have been a number of patients identified with dual diagnoses including the deletion and:

- Down syndrome
- Marfan syndrome
- CHARGE
- Neurofibromatosis
- Achondroplasia
- An *FGFR3* mutation (familial)
- Ehlers-Danlos syndrome (familial)
- Trisomy 8 mosaicism

22q deletion + Ehlers-Danlos
And, it is so common that there are affected first cousins by chance alone:

Parent of origin studies revealed the deletion occurred in the egg cells of the unrelated mothers

Saitta, 2004
Furthermore, with the advent of clinical arrays, detection is even higher:

- **Signature Genomics (Lisa Shaffer, PhD)** – has identified a 22q11.2 deletion in 1/143 patients referred for array based analysis whereas only 1/208 deletions would have been detected using FISH (~40,000 samples tested)

- **GeneDx (Sherri Bale, PhD)** – found 1/125 patients with a 22q11.2 deletion using oligo arrays but only 1/270 would have been detected using FISH (~6000 samples tested)

- **CHOP (Nancy Spinner, PhD)** – found 1/100 patients with a 22q11.2 deletion using SNP arrays as compared to 1/128 which would have been detected using FISH (~2000 samples)
Turning to deletion size, using FISH probes, most patients have been found to have the same size deletion (from A to D) which includes ~ 40 genes:

With \( TBX1 \) thought to be responsible for many of the phenotypic features including congenital heart disease.
With a subset of patients having a smaller deletion (A to B; A to C):

Vast majority of patients (~90%)
The importance of which is TBD as there is significant inter and intra familial variability including amongst identical twins regardless of the size of the deletion.

McDonald-McGinn, 2001

More about this later…
To date, the parent of origin does not appear to influence the clinical findings in familial or *de novo* cases

- VSD
- Learning Disability

-McDonald-McGinn, 2001-
The deletion size remains unchanged as it passes from parent to child.

So there is no evidence of “anticipation”
In the CHOP cohort, 94% of cases are *de novo*
However, as this is a contiguous deletion syndrome, affected individuals have a 50% recurrence risk.
And many only come to attention following the diagnosis in their more severely affected child

As seen in this mother and her son with a conotruncal cardiac anomaly
Moreover, in light of the:

• Declining infant mortality

• A modest effect on reproductive fitness

• And the 50% recurrence risk

It is expected that there will be ever increasing patient numbers, with resultant challenges to health care providers
It is additionally complicated in that an occasional parent will have somatic mosaicism as seen here:

Phenotypically normal father
Found to have mosaicism
- Initial study 13%
- Repeat study 12%
- Recurrence risk as high as 50%

Affected daughter
Furthermore, there have been reports of germ line mosaicism giving all non-deleted parents a small but important risk of recurrence.

Hatchwell, 1998; Sandrin-Garcia, 2002
Thus, we recommend 22q11.2 deletion studies in all parents because:

- A parent may have no symptoms of the deletion but have mosaicism or…
- An individual may have variable expressivity
In turning to the patient cohort, we have evaluated ~850 patients to date at the Children’s Hospital of Philadelphia (CHOP)
Males and females are equally likely to be affected

- Males: 49%
- Females: 51%
Unlike the early reports on patients with DiGeorge syndrome, mortality in our population is low.
In fact:

• Only 4% of patients have succumbed to complications associated with the deletion.

• The majority related to congenital heart disease.

• The median age of death at 4 months.
However, our patients are still relatively young, so time will tell if there is an effect on overall life expectancy:

- <1 year of age 2%
- between 1 and 5 years 11%
- between 6 and 10 years 21%
- between 11 and 15 years 26%
- >16 years 40%

As has been reported recently by Anne Bassett and her colleagues in Toronto
Morbidity, however, is more complex as we discuss the most common significant medical problems associated with the 22q11.2 deletion.
In our pediatric cohort these include:

- Immunodeficiency 77%
- Congenital Heart Disease 76%
- Palatal Differences 76%
- Hypocalcemia 49%
- Renal Abnormalities 36%
- Feeding/Swallowing 35%
- Learning Disabilities/MR > 95%
Specifically 77% of patients have immunodeficiency regardless of their clinical presentation including:

- Impaired T cell production 67%
- Humoral defects 23%
- IgA deficient 6%

- The majority of patients immune systems normalize by 1 year of age
- Although there is a tendency towards premature senescence of the immune system in our adult population
- With an increased incidence of upper respiratory infections and autoimmune disease

Cardiac defects are present in 76% of our patients including:

- Tetralogy of Fallot 20%
- Ventricular septal defect (VSD) 14%
- Interrupted aortic arch 13%
- Truncus arteriosus 6%
- Vascular ring 6%
- ASD/VSD 4%
- Atrial septal defect (ASD) 3%
- Other* 10%

* TGA, bicuspid aortic valve, pulmonary valve stenosis, isolated right pulmonary artery atresia, HLHS, aortic root dilatation, A-V canal, heterotaxy
Palatal abnormalities are present in 76% of patients including:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velopharyngeal incompetence (VPI)</td>
<td>42%</td>
</tr>
<tr>
<td>Submucosal cleft palate (SMCP)</td>
<td>16%</td>
</tr>
<tr>
<td>Overt cleft palate</td>
<td>11%</td>
</tr>
<tr>
<td>Bifid uvula</td>
<td>5%</td>
</tr>
<tr>
<td>Cleft lip/palate</td>
<td>2%</td>
</tr>
<tr>
<td>Suspected VPI/requiring follow-up</td>
<td>9%</td>
</tr>
</tbody>
</table>

But most will either not be identified in infancy or repaired at that time and therefore young children require long term surveillance.
Velopharyngeal port as seen during nasendoscopy:

Patient without VPI

At rest

During crying/swallowing

Patient with VPI

At rest

During crying/swallowing

Photos courtesy of Dr. Peter Randall
Hypocalcemia is present in 49% of patients which:

• Often resolves in the newborn period

• May recur during times of illness or stress such as during puberty

• May present initially in adolescence or adulthood
### Genitourinary abnormalities are present in 36% of patients:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural urinary tract anomaly</td>
<td>31%</td>
</tr>
<tr>
<td>Dysfunctional voiding</td>
<td>11%</td>
</tr>
<tr>
<td>Unilateral renal agenesis</td>
<td>10%</td>
</tr>
<tr>
<td>Multicystic/dysplastic kidney</td>
<td>10%</td>
</tr>
<tr>
<td>Echogenic/hypoplastic kidneys</td>
<td>6%</td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>5%</td>
</tr>
<tr>
<td>Duplex kidney</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Hypospadias</td>
<td>8%</td>
</tr>
<tr>
<td>Undescended testes</td>
<td>6%</td>
</tr>
<tr>
<td>Absent uterus</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

Wu, et al., 2002; Sundaram, 2007
Feeding and swallowing abnormalities are found in 35% of patients including:

- Gastroesophageal reflux
- Esophageal dysmotility
- Constipation
- Prolonged tube feedings
- G-tube placement

These are some of the most difficult problems are families to deal with

Nasopharangeal Reflux

Esophageal dysmotility

Eicher, 1997
However, many rare problems also contribute to significant morbidity in our patients.
These include:

- Laryngotracheo-oesophageal abnormalities such as:
  - Vascular Ring
  - Laryngeal Web
  - T-E fistula or esophageal atresia

- Gastrointestinal differences such as:
  - Intestinal malrotation
  - Hirshsprung’s disease

- Diaphragmatic hernia
  - including congenital
  as well as late presentations
- Cervical spine anomalies – occasionally leading to cord compression

- Unprovoked Seizures

- Polymicrogyria

- Autoimmune Disease – Juvenile Rheumatoid Arthritis, Idiopathic Thrombocytopenia, Autoimmune Neutropenia, and Graves Disease
• **Skeletal Abnormalities**
  - scoliosis
  - butterfly vertebrae
  - craniosynostosis
  - polydactyly

• **Bernard-Soullier syndrome** (Platelet abnormalities)

• **Growth Hormone Deficiency**

• **Hearing Loss** – sensorineural and conductive

• **Ophthalmologic Differences**
  - Scleracornea
  - Coloboma

• **Occasional Neoplasias**
  - Hepatoblastoma (0.3% compared to 1.5/1 million)
  - Wilms tumor/Neuroblastoma/Renal cell carcinoma
In terms of neuropsychological issues, the patients can be broken down into two groups: Preschool and School aged.

For young children we’ve found:
- Motor milestone delays (mean age at walking - 18 months)
- Delays in emergence of language (many non-speakers at age 2 & 3 years)
- Frank autism or autistic like features (~ 10%)

But all benefit from early intervention strategies!

Gerdes, 2001; Solot, 2001; Fine, 2004
For school aged children using the age appropriate Weschler IQ battery:

<table>
<thead>
<tr>
<th></th>
<th>Full Scale IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average range</td>
<td>18%</td>
</tr>
<tr>
<td>Low average range</td>
<td>20%</td>
</tr>
<tr>
<td>Borderline range</td>
<td>32%</td>
</tr>
<tr>
<td>Mentally retarded range</td>
<td>30%</td>
</tr>
</tbody>
</table>

But the Full Scale IQ generally does not reflect the individual’s cognitive abilities

Moss, 1999; Woodin, 2001
Patient #9117

Full scale IQ = 87*
Verbal IQ = 111
Performance IQ = 65

46 point split

*invalid
Thereafter we found:

• A >12 point split between the verbal and performance IQ in 65% of patients

• Therefore, full scale IQs do not accurately represent many of these patients and their verbal and performance IQs should be considered separately

• This information has direct ramifications for cognitive remediation in this population
In Terms of Psychiatric Findings

• We have not found the 25% incidence of schizophrenia which has been reported by others in our own cohort.

• This may be related to our ascertainment bias or the age distribution of our patients.

• However, we have found a high incidence of anxiety, ADHD, and obsessive compulsive behaviors such as perseverations.
Turning to our adult population (N = 42)

- 70% of individuals graduate secondary school
- 30% require learning support

McDonald-McGinn, 2001
But despite the 70% graduation rate affected parents have:

- More difficulty understanding the ramifications of the diagnosis and in complying with treatment recommendations

- A poor understanding of their recurrence risk

- Generally require a social safety net, often in the form of a social worker or unaffected relative
Occupations include:

- Chef
- Farmer
- Security guards
- Maintenance workers
- Office workers
- Delivery Persons
- Homemakers

McDonald-McGinn, 2001
Here too there is significant variability

As we have followed several young adults identified in adolescence:

• Several are enrolled in college

• Some have passed the college entrance exam

• One graduated with a 3.0 Grade Point Average and immediately secured an Early Childhood Education Position

In addition, we have an adult with a Master’s Degree in Family Therapy and she has a thriving private practice
So, knowing all this about patients with a standard deletion and since the advent of clinical arrays, what do we know about non-deleted patients with typical phenotypic features of the 22q11.2 deletion?
We know that some of these patients actually have other well described diagnoses such as:

• **CHARGE syndrome** (*CHD7* mutations)
  - Congenital heart disease
  - Immune deficiency
  - Hypocalcemia

• **Goldenhar syndrome**
  - Congenital heart disease
  - Renal anomalies
  - Butterfly vertebrae

• **Kabuki syndrome**
  - Congenital heart disease
  - Cleft palate
  - Butterfly vertebrae
And some patients with a subset of typical features of the 22q11.2 deletion have mutations in the *TBX1* gene within the 22q11.2 deleted region:

Yagi, 2003; Zweier, 2007; Torres-Juan, 2007
While others have described patients with atypical 22q11.2 deletions who would have been missed using standard FISH studies.
At CHOP we’ve identified 17 such patients with an atypical 22q11.2 deletion

Of these:

- 2 had proximal deletions including \( TBX1 \)
- 13 had B-D deletions
- 2 had C-D deletions
- 4 were parents of affected individuals (all B – D)
So, 15/17 atypical deletions did not include *TBX1* and 13/17 patients had B–D deletions.

- **N = 2**
  - D22S75 (N25) *
  - HIRA
  - UF144L
  - CDC45L
  - TBX1

- **N = 13**
  - ZNF74

- **N = 1**
  - CDC45L

- **N = 1**
  - **Standard Large 3 Mb deletion**

- **Variant 1.5 Mb deletion**
The facial features in these patients are variable
As compared with patients who have the standard deletion:

- Hooding of the eyelids
- Bulbous nasal tip with hypoplastic alae nasae
- Nasal dimple
- External ear differences
And the clinical features are also variable:

<table>
<thead>
<tr>
<th>Deletion Size</th>
<th>Heart</th>
<th>Palate</th>
<th>Developmental Delay/MR</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Including <em>TBX1</em></td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
<td>Hypocalcemia, OCD, Anxiety</td>
</tr>
<tr>
<td>B-D</td>
<td>4/13*</td>
<td>3/13</td>
<td>8/13</td>
<td>Absent kidney, Hypocalcemia, Polymicrogyria, GERD (3), Short stature/GHD (3), Laryngeal web, Iris coloboma, Preauricular tags</td>
</tr>
<tr>
<td>C-D</td>
<td>0/2</td>
<td>1/2</td>
<td>2/2</td>
<td>Hypocalcemia, OCD/Tourette’s</td>
</tr>
</tbody>
</table>

* TOF, VSD/coarctation of aorta, IAA, truncus arteriosus
However, there are interesting trends such as:

- Palatal anomalies (6/17)
- Gastrointestinal Problems (3/17)
- Short Stature/GHD (3/17)
- Hypocalcemia (3/17)
- Developmental delay (12/17)
- Cardiac Differences (5/17) including abnormalities typically associated with the standard deletion (TOF, IAA, and Truncus arteriosus as seen in 3 patients with a B – D deletion)
In turning our attention to 22q11.2 duplications
In looking at prevalence:

- **Signature Genomics (Lisa Shaffer, PhD) –** has identified a 22q11.2 duplication in 1/200 patients referred for array analysis.

- **GeneDx (Sherri Bale, PhD) –** found 1/270 patients with a 22q11.2 duplication using oligo arrays.

- **CHOP (Nancy Spinner, PhD) –** found 1/500 patients with a 22q11.2 duplication using SNP arrays.

So, as has been reported in the literature, the 22q11.2 duplication is detected approximately half as frequently as the 22q11.2 deletion on average.
At CHOP we have evaluated 16 such patients with a 22q11.2 duplication

Of these:

• 7 had standard A – D duplications

• 9 had smaller atypical duplications

• 6 were parents of affected children – with both standard and atypical duplications
Here too the patients facial features are variable
And like the patients with 22q11.2 deletions, their clinical findings are variable as well.

<table>
<thead>
<tr>
<th>Duplication Size</th>
<th>Heart</th>
<th>Palate</th>
<th>Developmental Delay</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - D</td>
<td>1/7</td>
<td>1/7</td>
<td>3/7 *</td>
<td>Seizures, GERD (3), Chronic URI’s, Hypocalcemia, Microcephaly, Macrocephaly, Tracheal stenosis, Preauricular pits, Hearing Loss</td>
</tr>
<tr>
<td>Atypical</td>
<td>2/9</td>
<td>0/9</td>
<td>6/9</td>
<td>GERD (2), Microcephaly, Macrocephaly, Seizures</td>
</tr>
</tbody>
</table>

* One patient died in infancy
Of note, as compared with the literature:

- We too had a high incidence of familial cases (where 6/7 cases were familial where both parents were available for testing)

- Although we had a relatively low incidence of palatal anomalies (1/16)

- And most patients came to clinical attention due to developmental disabilities or they might otherwise have gone undetected
Thus, this new cohort of patients with atypical 22q11.2 deletions and to some extent duplications serves to highlight the fact that:

- Some patients with typical clinical features may in fact have smaller deletions or duplications which will require alternative methods of identification such as CGH, MLPA, or whole genome array.

- Furthermore, such patients will likely be key in continuing the search for genotype–phenotype correlations.

- And clinical evaluations in these patients should mirror what we do for those patients with the standard deletion until proven otherwise.
Turning to Clinical Evaluations:

Based on our International Consortium, which met first in Marseille in 2006 and then again in Utrecht in 2008, we have developed clinical practice guidelines for the 22q11.2 deletion, the highlights of which include:

• Standard initial assessment and work-up for all ages including Cardiology, Endocrinology, Speech/Language/Developmental Assessments

• Renal Ultrasound/C-spine radiographs

• Parental deletion studies

• Individualized work-up depending on symptomatology
Which may include one or more of the following:

- Plastic Surgery/ENT
- Dentistry
- Immunology/Rheumatology
- Urology/Nephrology
- Orthopaedics
- General Surgery
- Ophthalmology
- Gastroenterology/Feeding Team
- Hematology
- Neurology/Neurosurgery
- Psychiatry
This discussion will be refined further

As the 7th International 22q11.2 Deletion Syndrome Conference entitled

“Treatment as We Move Into the New Decade”

Will be held in Coventry, England

July 29th – 31st, 2010

This will feature back – to – back parent and professional meetings

A Call for Abstracts will be forthcoming

For more information please go to:

www.maxappeal.org.uk
Based on this information we recognize:

- Clinical genome wide arrays are helping us to define complex phenotypes in this region.

- So the breadth of our clinical experience will give us a window on complimentary genetic mechanisms and/or position affects.

- And most importantly our collective clinical experience will help us to better manage these patients.
No where will this information be more important than in the prenatal setting as such sensitive studies move further into the clinical arena.
In the meantime prenatal clues to the diagnosis of 22q11.2 aneuploidies in the general population include:

1. Congenital heart disease where 22q11.2 deletions have been observed in:
   - 52% of patients with IAA Type B
   - 34% of patients with Truncus arteriosus
   - 16% of patients with tetralogy of Fallot

Goldmuntz, 1998
As well as other abnormalities, which when present on prenatal ultrasound, especially in the face of congenital heart disease, may herald a 22q11.2 deletion including:

2. Polyhydramnios 16%
3. Polydactyly 6%
4. Cleft lip and/or palate 2% - 11%
5. Congenital Diaphragmatic Hernia 1%
6. Spina bifida <1%
7. Craniosynostosis <1%
For those families where there is a 50% recurrence risk, prenatal diagnostic options include:

- Level II Ultrasound (~16 weeks)
- Fetal Echocardiography (>18 weeks)
- Chorionic Villus Sampling (12 weeks)
- Amniocentesis (16 weeks)
As well as:

Donor Egg or Sperm
And Preimplantation Genetic Diagnosis (PGD)

Genetic testing is performed on a single blastocyst.

Embryos without the genetic defect are implanted.
So in summary, based on what we have observed thus far in patients with the standard 22q11.2 deletion, atypical deletions and 22q11.2 duplications:

• We will continue to evaluate patients with atypical deletions and duplications as we do those with a standard 22q11.2 deletion until proven otherwise

• And we will counsel families in the prenatal setting with caution until additional data becomes available
Thank you for your kind attention and many thanks to my collaborators

Elaine H. Zackai, MD
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Patrick Pasquariello, MD
Peter Randall, MD
Patricia Schultz, MSN
Cynthia Solot, MA, CCC
Kathleen Sullivan, MD, PhD
And many thanks to the children and their families for everything that they’ve taught us!

“It always starts with a patient”!