Copy Number Analysis in Human Disease-Part II (Clinical Application)

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Outline

- Introduction
- Case presentation
- The clinical application of microarray analysis
- Conclusions
Introduction

Chromosomal abnormalities are an underlying cause of:

- Congenital anomalies
- Dysmorphism
- Global developmental delay (GDD) and intellectual disability (ID)
- Autism and other neurobehavioral phenotypes
- IUGR and FTT
- Miscarriages and infertility
- Several other genetic syndromes
Genomic Disorders

- Genomic disorders are clinical phenotypes caused by abnormal dosage or dysregulation of one or more genes resulting from rearrangement of the genome.

Non-allelic homologous recombination

Gain/duplication

Loss/deletion

Cross over between A & D
Molecular Mechanisms for Genomic Disorders

- Gene dosage
- Gene Interruption
- Positional effect
- Gene fusion
- Unmasking recessive allele or imprinting
Traditional Cytogenetic Methods

- Giemsa stained metaphase chromosomes identifies balanced and unbalanced structural and numerical chromosomal abnormalities.
Traditional Cytogenetic Methods
Fluorescence in situ Hybridization (FISH)

Denatured chromosomes

Label → Denature → Anneal
Case Presentation-HPI

- 8 yo patient with obesity, “distractible behavior” and speech delay.
- “Hungry all the time.”
- 1 yo at 50th centile for wt.
- She started gaining excessive wt during the 2nd year of life and since then she is consistently above the 95th percentile for wt.
- Speech delay & therapy
- “Difficulties with math”
- “Easily distractible”
Case Presentation-PMH

- The only child for 45 yo father and 44 yo mother.
- Parents are healthy and both completed college. Mother’s BMI-28
- No Fx of GDD/ID, Sz, MCA, morbid obesity, or consanguinity.
Case Presentation-Fx & Sx

- Term, AGA
- AMA→amniocentesis (nl)
- Tonic-clonic seizures began at 6 mo
- Controlled with phenobarbital which was weaned and discontinued at 2.5 years of age.
Case Presentation

- BMI: 30.9 kg/m², → 27.85 (endocrine clinic)
- Ht- 70th, OFC-55.7 cm (+3 SD)
- Shortened neck, mild overbite, post. rotation of ears, bilateral 2-3 cutaneous syndactyly, hyperextensible fingers
Case Presentation

- EEG –nl. Brain MRI- Chiari I malformation
- Renal us & echo-nl
- FT₄/TSH, lipid panel, HbA₁c-nl
- Karyotype, PWS methylation-nl
- CMA-~590 Kb deletion on 16p11.2 (29,508,381-30,099,396)
Copy Number Variations (CNVs)

- CNV is a DNA segment (>1 Kb) with a variable copy number compared with a reference genome “pathogenic”; “benign”; “of unclear significance”
- Genomes of unrelated individuals are different in 12% of the loci.

Risk assessment of CNV of unclear significance:
1. *De novo* or inherited from an affected parent
2. Reported in association with a disease vs. database of healthy individuals
3. The gene content of the region
4. Loss>gain
5. Data bases
<table>
<thead>
<tr>
<th>Primary Criteria</th>
<th>Indicates CNV Is Probably:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pathogenic</td>
</tr>
<tr>
<td>1. a. Identical CNV inherited from a healthy parent\textsuperscript{b}</td>
<td>![Checkmark]</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Expanded or altered CNV inherited from a parent</td>
<td>![Checkmark]</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>c. Identical CNV inherited from an affected parent</td>
<td>![Checkmark]</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2. a. Similar to a CNV in a healthy relative</td>
<td>![Checkmark]</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Similar to a CNV in an affected relative</td>
<td>![Checkmark]</td>
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<td></td>
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<tr>
<td>3. CNV is completely contained within genomic imbalance defined by a high-resolution technology in a CNV database of healthy individuals</td>
<td>![Checkmark]</td>
</tr>
<tr>
<td></td>
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<tr>
<td>4. CNV overlaps a genomic imbalance defined by a high-resolution technology in a CNV database for patients with ID/DD, ASD, or MCA</td>
<td>![Checkmark]</td>
</tr>
<tr>
<td></td>
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<tr>
<td>5. CNV overlaps genomic coordinates for a known genomic-imbalance syndrome (i.e., previously published or well-recognized deletion or duplication syndrome)</td>
<td>![Checkmark]</td>
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<tr>
<td></td>
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<tr>
<td>6. CNV contains morbid OMIM genes\textsuperscript{c}</td>
<td>![Checkmark]</td>
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<td></td>
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<tr>
<td>7. a. CNV is gene rich</td>
<td>![Checkmark]</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>b. CNV is gene poor</td>
<td>![Checkmark]</td>
</tr>
</tbody>
</table>
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"Forward Genetics" or "Genomics"

- **Forward genetics** refers to a process where studies are initiated to determine the genetic basis of observable phenotypic variation.
“Reverse Genetics” or “Genomics”

- **Reverse genetics** refers to the study of phenotypes associated with a specific genetic change. It enables clinical characterization of previously unknown genomic disorders.
CMA and Congenital Anomalies

PMID: 25205790

- Cytogenetic abnormalities are a major cause of multiple congenital anomalies (MCA), especially when they are associated with FTT, or GDD, malformations affecting a second organ or DFs (Am. J. Med. Genet. 12 (1982), pp. 155-173).

Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cause of congenital heart defects in selected patients European Heart Journal (2007) 28, 2778-2784

- 30% of patients with CHDs associated with other malformations, DF or learning disabilities/ID and in whom the karyotype analysis was normal, carried pathogenically significant genomic imbalances
De novo copy number variants are associated with congenital diaphragmatic hernia

- Investigated the frequency of chromosomal anomalies and copy number variants (CNVs) in 256 parent–child trios of CDH
- Identified chromosomal anomalies in 16 patients (6.3%) of the series including three aneuploidies, two unbalanced translocation, and 11 patients with de novo CNVs ranging in size from 95 kb to 104.6 Mb.

Evaluation of GDD/ID by Microarray

- **A diagnostic challenge:** cause is unknown in more than one-half of the cases (*Pediatrics* 117 (2006), pp. 2304–2316)

- **Banded chromosome analysis:** ~3.5-4%; subtelomere FISH **2.5-7%** (*Neurology* 60 (2003), pp. 367–380; *J. Med. Genet.* 43 (2006), pp. 478–489)

- **A meta-analysis** of previously reported aCGH studies and the analysis of 140 additional patients with idiopathic ID (total of 432 patients) showed that **20%** of patients have genomic imbalances, **11%** have subtelomeric rearrangements (*J Med Genet* 2006; 43, 625–633).
Evaluation of GDD/ID by Microarray

- A review of 29 studies of DD/ID patients showed a yield of 19% pathogenic aberrations (aCGH was used as the initial genetic test) (European Journal of Medical Genetics 52 (2009) 161–169)

- The recurrence rate of chromosomal imbalances was very low:
  - Genetic heterogeneity
  - The need for whole-genome methods.
Evaluation of Autism by Microarray

- Using current microarrays, relevant \textit{de novo} genomic imbalances can be identified in $7\%$–$20\%$ of ALL individuals with autism of unknown cause (http://www.ncbi.nlm.nih.gov/books/NBK1442/).

- Clinically relevant CNVs were identified in $28\%$ of individuals with \textit{syndromic autism} (J Med Genet. 2006;43:843–9.)

- CNVs were found in $5\%$–$8\%$ of cases of simplex high-functioning autism (Neuron. 2011 Jun 9;70(5):863-85.)
The Causes of Autism According to Genetic Contribution

- **CNVs**: 7-20%
- **Single-gene disorders**: 5-7%
- **Metabolic disorders**: <5%
- **Unknown causes**: >70%
Cytogenetic microarray (CMA) testing for copy number variation (CNV) is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:

- A. Multiple anomalies not specific to a well-delineated genetic syndrome
- B. Apparently non-syndromic developmental delay/intellectual disability
- C. Autism spectrum disorders
- Small deletions of a single or few dosage-sensitive genes
- Translocation breakpoints within dosage-sensitive genes
- The genetic basis of different genetic conditions has been identified using aCGH.
- Examples: CHARGE, Goltz syndrome (focal dermal hypoplasia)

Mutations in PORCN gene were identified
Patient Hx: Clinical Features

Patient exhibits ALL 8 major PWS diagnostic criteria:

- Neonatal and infantile hypotonia
- Feeding problems in infancy
- Excessive weight gain after 12 months (~63.3 kg; >>97th percentile); morbidly obese at 4-4/12 yrs
- Bitemporal narrowing and almond-shaped eyes
- Hypogonadism-small external genitalia & hypoplastic scrotum
- Small hands and feet relative to body size
- Hyperphagia/food seeking behavior
- Mild ID; meets criteria for a diagnosis of autism.

Deficiency of HBII-85 snoRNAs causes the key characteristics of the PWS phenotype.
Mosaicism: The presence of more than one genetically distinct somatic cells in a single organism

Low-level mosaicism can be missed by conventional chromosome analysis if the specimen is masked by a high percentage of normal cells

The culturing and the stimulation by a mitogen of T lymphocytes needed in the conventional chromosome analysis introduces a selection bias against the abnormal cell line

Clinical Features:
- Growth restriction
- GDD
- Dysmorphism
- Body asymmetry
- Pigmentary skin changes
- Genital and cardiac anomalies
• In 3 patients, the initial chromosome analysis was normal.

• Higher level of mosaicism were detected in tests performed on whole blood (array-CGH and interphase FISH) than tests performed on PHA-stimulated lymphocytes.
Identification of Incestuous Parental Relationships by SNP-based DNA Microarrays

- SNP arrays can identify regions of absence of heterozygosity (homozygosity).
- Uniparental disomy or identity by descent.
- Disabilities are frequent in children born of incestuous parentage.
- They raise important legal and ethical concerns.
- If the mother is minor: report to child protection services

Figures courtesy of Dr. Shashikant Kulkarni.
A recurrent 1.5-Mb microdeletion syndrome

- Mild to moderate ID (9/9)
- Epilepsy and/or abnormal EEG (7/9)

All affected individuals also had mild facial dysmorphism (hypertelorism, upslanting palpebral fissures, prominent philtrum with full everted lips, short and/or curved fifth finger and short fourth metacarpals).
15q13.3 Microdeletion Syndrome

**CHRNA7**: Cholinergic receptor, nicotinic, alpha 7

**Class I**
- BP1
- BP2
- NIP1A
- NIP2A
- CVITP1
- GOP5
- MKRN3
- MAGEL2
- NDN
- SNRPN
- HBITI-85
- HBITI-52
- UBE3A

**Class II**
- BP3
- BP4
- BP5
- GABRB3
- GABRG3
- OCA2
- APBA2
- TJP1

**PWS/AS Critical Region**
- MTMR15
- MTMR10
- TRPM1
- KLF13
- OTUD7A
- CHRNA7
<table>
<thead>
<tr>
<th>Study</th>
<th>Clinical Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>J Med Genet. 2009 Apr 21.</td>
<td>ID, hyperactivity, aggressive and impulsive behavior</td>
</tr>
</tbody>
</table>

~73% of 15q13.3 deletions are inherited
A Small Recurrent Deletion within 15q13.3 is Associated with a Range of Neurodevelopmental Phenotypes

(Nat Genet 2009; 41:1269-1271)

The entire CHRNA7 gene and the first exon of one of the isoforms of OTUD7A
### Table 1: Phenotypic features of ten individuals from four unrelated families with a 680-kb deletion within chromosome 15q13.3

<table>
<thead>
<tr>
<th></th>
<th>Pt 1</th>
<th>Pt 2 (brother of 1)</th>
<th>Pt 3 (sister of 1)</th>
<th>Pt 4 (mother of 1)</th>
<th>Pt 5 (maternal aunt of 1)</th>
<th>Pt 6 (MGM of 1)</th>
<th>Pt 7</th>
<th>Pt 8 (mother of 7)</th>
<th>Pt 9</th>
<th>Pt 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>8 yr</td>
<td>4 yr</td>
<td>3 yr</td>
<td>30 yr</td>
<td>21 yr</td>
<td>52 yr</td>
<td>21 mo</td>
<td>23 yr</td>
<td>16 yr</td>
<td>8 mo</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Ancestry</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>AA</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Seizures/EEG</td>
<td>None/abnormal EEG</td>
<td>None</td>
<td>None</td>
<td>Absence epilepsy</td>
<td>Generalized epilepsy since childhood</td>
<td>None</td>
<td>None</td>
<td>Epilepsy since 5 yr</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Inheritance of CHRNA7 del.</td>
<td>Mat</td>
<td>Mat</td>
<td>Mat</td>
<td>Mat</td>
<td>Mat</td>
<td>Unkn</td>
<td>Mat</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Unkn</td>
</tr>
<tr>
<td>Other CMA abnormal</td>
<td>None</td>
<td>Unkn</td>
<td>Unkn</td>
<td>None</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Xq26.2 dup</td>
<td>Unkn (not pat)</td>
<td>Unkn</td>
<td>Unkn</td>
</tr>
</tbody>
</table>

### Diagram

- **CHRNA7 deletion**
- 52 yo, MR, MS
  - 30 yo, MR, absence sz
  - 21 yo, MR, epilepsy
  - 8 yo, severe MR, abnl EEG
  - 4 yo, DD, hypotonia
  - 3 yo, GDD
A 39 yo male with 15q13.3DS: epilepsy, ID, psychosis, and recurrent episodes of aggressive rage.

Treatment with the NChR allosteric modulator and acetylcholinesterase inhibitor, galantamine, led to a dramatic decline in the frequency and intensity of rage outbursts
In 2008, a *de novo* recurrent ~600 Kb deletion and a *de novo* or inherited reciprocal duplication on 16p11.2 were found in ~1% of autism subjects and ~1.5% of children diagnosed with developmental or language delays (N Engl J Med. 2008;358:667–75.)
A total of 27 (17) deletion cases and 18 (10) duplication cases were detected with a frequency of \(~0.6\%\) in samples submitted to a diagnostic laboratory.
All Rearrangements were Recurrent (~600 Kb)
Phenotypic Characterization of Patients with Deletion or Duplications of 16p11.2

Broad forehead, micrognathia, hypertelorism, and a flat midface. The broad forehead, macrocephaly and flat midface give these patients a distinct facial gestalt.
Duplication patients were more grossly dysmorphic as compared to the deletion patients, but there was no recognizable pattern for these features.
Deletions and Duplications of 16p11.2 are Associated with an Abnormal Head Size

- 2/3 of deletion pts had absolute or relative macrocephaly
- The duplication patients had small mean head sizes with 6/10 having microcephaly
Increased incidence of congenital anomalies in individuals with the 16p11.2 deletion (30%) or duplication (50%).

Congenital diaphragmatic hernia, chordae, hypospadias, cleft palate, polydactyly, congenital heart defect, multicystic dysplastic kidney, fusion of ribs, scoliosis, vertebral anomalies, and pyloric stenosis (3 cases).

Brain MRI or CT were abnormal 9/15 patients (prominent CSF spaces (5/15), white matter changes/gliosis (3/15), Chiari I malformation (1/15), and abnormal corpus callosum (1/15).
16p11.2 Syndrome and Other Phenotypes

- 2 deletion pts have cervicothoracic syringomyelia and 1 duplication pt had long thoracolumbar syringomyelia.
- All 3 patients displayed lower extremity spasticity and gait disturbance.
- TBX6, a transcription factor important in developmental processes, is a candidate gene.

European Journal of Human Genetics (2011) 19, 152-156
16p11.2 Syndrome and Seizures

- 38% and 30% patients with the deletion and duplication, respectively.

- The seizures typically start during the first year of life, are easily controlled with antiepileptic medications, and tend to resolve or decrease in severity during childhood.
The incomplete penetrance and variable expressivity complicate both the clinical interpretation of the molecular data and the genetic counseling.

The phenotypic variability may be related to:
- Variants on the remaining hemizygous allele
- Imprinting effect
- Different genotypes elsewhere in the genome
- sex- and age-dependent penetrance for specific traits
16p11.2 Deletions Associated with Severe Early-Onset Obesity [Nature 2010; 463(7281):666–670]

- 9/312 subjects with congenital malformations and/or DD and obesity had the 16p11.2 deletion (2.9%)
- Among cohorts with obesity:

<table>
<thead>
<tr>
<th>Deletions/Total</th>
<th>Lean/normal</th>
<th>Overweight</th>
<th>Obese</th>
<th>Morbidly obese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/7,434</td>
<td>0/4,254</td>
<td>3/1,752</td>
<td>13/2,260</td>
</tr>
</tbody>
</table>

- The obesity phenotype is significant in adults (age-dependent penetrance).
Reciprocal Changes in Gene Dosage at 16p11.2 Result in Mirror Phenotypes

<table>
<thead>
<tr>
<th>16p11.2 Deletion</th>
<th>16p11.2 Duplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>Schizophrenia/ADHD</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>Microcephaly</td>
</tr>
<tr>
<td>Hyperphagia</td>
<td>Anorexia</td>
</tr>
<tr>
<td>Overweight</td>
<td>Underweight</td>
</tr>
<tr>
<td>CNV locus</td>
<td>Condition</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>1q21.1</td>
<td>Autism</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>16p11.2</td>
<td>Autism</td>
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<tr>
<td></td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>22q11.21</td>
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</tr>
<tr>
<td></td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>22q13.3</td>
<td>Autism</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia</td>
</tr>
</tbody>
</table>

Data from studies of head and brain size phenotypes indicate that autism is commonly associated with enhanced brain growth, whereas schizophrenia is characterized, on average, by reduced brain growth.
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Conclusions-I

- CMA is more efficient and comprehensive diagnostic tool than conventional cytogenetics.
- It has revolutionized the diagnostic work-up of patients with GDD/ID, MCA, autism, dysmorphism.
- It is a powerful tool in disease gene discovery and in deciphering the genomic basis of many novel chromosomal rearrangement syndromes.
‘Reverse genomics’ enables clinical characterization of previously unknown genomic disorders.

The emerging phenotypes of novel genomic disorders may shed light on the genetic basis of common conditions.
THANK YOU