Demonstrating Clinical Research Utility:
A Custom Microarray to Study Neurodevelopmental Disorders

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Nashville, TN
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Disclosure

Dr. Shen is employed by Boston Children’s Hospital and is a consultant for Claritas Genomics. Claritas Genomics is an affiliate of Boston Children’s Hospital.
Outline

• Association of copy number variants with neurodevelopmental disorders

• From whole genome array to custom whole genome array-the design principles

• What does the custom array detect that routine arrays do not?

• What is the role for this CMA in the NGS era?
What Are Neurodevelopmental Disorders (NDD)

DSM-5

Neurodevelopmental Disorders

- Intellectual Disabilities
- Communication Disorders
- Autism Spectrum Disorder
- Attention-Deficit/Hyperactivity Disorder
- Specific Learning Disorder
- Motor Disorders
- Other Neurodevelopmental Disorders
Neurodevelopmental Disorders Are Highly Genetic

Heritability
ASD 80%
ADHD 79%
DCD 70%
Tic syndrome 56%

Comorbidity with ASD
ADHD 51.1%
DCD 30.3%
Tic disorder 44.7%
Learning disability 34.5%

Copy Number Variants: Common Causes of NDD

Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH)
J Schoumans, C Ruivenkamp, E Holmberg, M Kyllerman, B-M Anderlid, M Nordenskjöld


Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis
Nigel M Williams, Irina Zaharieva, Andrew Martin, Kate Langley, Kiran Mantripragada, Ragnheidur Fossdal, Hreinn Stefansson, Kari Stefansson, Pall Magnusson, Olafur O Gudmundsson, Omar Gustafsson, Peter Holmans, Michael J Owen, Michael O'Donovan, Anita Thapar

Strong Association of De Novo Copy Number Mutations with Autism
Jonathan Sebat et al.
Science 316, 445 (2007);
DOI: 10.1126/science.1138659
Whole Genome Microarray (244k) Led To The Discovery Of Novel NDD Loci Such As 16p11.2
Association Between 16p11.2 Imbalances And Autism

~0.01% in general population
~0.1% in individuals with psychiatric or language disorders
~1% in autism patients
Evidence Suggests Microarray As First Line Test For ASD

CMA has the highest detection rate among clinically available genetics tests for patients with ASD. ... CMA should be considered as part of the initial diagnostic evaluation of patients with ASD. *Pediatrics* 2010, April.
“Our recommendation based on current evidence is to offer CMA as the first-tier test, in place of G-banded karyotype, for patients with unexplained DD/ ID, ASD, or MCA.”
Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

Melanie Manning, MD, MS FACMG and Louanne Hudgins, MD, FACMG

Recommendations:

1. 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

2. Further determination of the use of CMA testing for the evaluation of the child with growth retardation, speech delay, and other less-well studied indications is recommended, particularly via prospective studies and aftermarket analysis.

3. Appropriate follow up is recommended in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling.
Rationale For Redesigning The Array

- Clinical relevance differs in different regions of the human genome
- Both large genomic imbalances and small intragenic CNVs contribute to NDD
- The vast majority of CNVs are population polymorphisms and clinically benign
- Long contiguous stretches of homozygosity (LCSH) may be clinically significant
- On going effort to continue improving clinical sensitivity and specificity, as well as cost-effectiveness
The Basis For Our Custom Design

• >10,000 pediatric patients examined at BCH/Claritas, the majority of patients were phenotypically characterized as having one or multiple neurodevelopmental disorders

• Better understanding of the old and new genomic disorders

• Better understanding of the genes and pathways involved in neurodevelopmental disorders

• Better understanding of the probe behavior

• Capability of genotyping using the Agilent array
The ClariView Array: 4x180K SNP+CNV Array

Chromosome 2

NRXN1
Unique Design Features Of The Claritas Clariview Array

1. **ClariView Array** detects both CNV and AOH
   - 30K SNP probes: Sufficiently detect AOH > 7Mb.
   - 30K additional CNV probes.

2. ~1000 NDD genes with enhanced coverage.

3. Reduced probe coverage in clinically irrelevant copy number polymorphic regions.
Raw Data: Genome View of A Normal Case
Raw Data: A Case With 15q11.2 Triplication
A whole FOXG1 gene deletion (red bar) would be missed by an off-the-shelf design.
Array Design Detects Small Intragenic CNVs

Minimum size: 7kb

Maximum size: 34kb
Detecting Functional Non-Coding Small CNVs

4kb deletion detected in promoter region of the YWHAHE gene.
# Genes Covered By > 5 Probes

## Diseases and Disorders

<table>
<thead>
<tr>
<th>Name</th>
<th>p-value</th>
<th># Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>2.02E-100 - 3.43E-08</td>
<td>2100</td>
</tr>
<tr>
<td>Gastrointestinal Disease</td>
<td>9.23E-90 - 2.77E-08</td>
<td>1474</td>
</tr>
<tr>
<td>Developmental Disorder</td>
<td>1.37E-47 - 3.65E-08</td>
<td>877</td>
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<tr>
<td>Neurological Disease</td>
<td>1.37E-47 - 3.65E-08</td>
<td>1053</td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td>5.77E-45 - 2.04E-08</td>
<td>515</td>
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</table>

## Physiological System Development and Function

<table>
<thead>
<tr>
<th>Name</th>
<th>p-value</th>
<th># Molecules</th>
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<tbody>
<tr>
<td>Organismal Survival</td>
<td>1.68E-67 - 7.05E-25</td>
<td>878</td>
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<tr>
<td>Nervous System Development and Function</td>
<td>3.50E-52 - 3.73E-08</td>
<td>908</td>
</tr>
<tr>
<td>Embryonic Development</td>
<td>4.49E-43 - 4.13E-08</td>
<td>834</td>
</tr>
<tr>
<td>Organ Development</td>
<td>4.49E-43 - 3.89E-08</td>
<td>674</td>
</tr>
<tr>
<td>Organismal Development</td>
<td>4.49E-43 - 3.89E-08</td>
<td>1006</td>
</tr>
</tbody>
</table>
Little Or No Coverage In Common Polymorphic CNVs

Probe distribution track

Copy number polymorphism track
## Genes Covered by < 5 Probes

### Diseases and Disorders

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<tr>
<th>Name</th>
<th>p-value</th>
<th># Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>2.50E-47 - 4.02E-03</td>
<td>6764</td>
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<tr>
<td>Infectious Disease</td>
<td>1.76E-24 - 3.28E-03</td>
<td>1983</td>
</tr>
<tr>
<td>Dermatological Diseases and Conditions</td>
<td>2.60E-14 - 3.50E-03</td>
<td>935</td>
</tr>
<tr>
<td>Immunological Disease</td>
<td>3.86E-13 - 2.72E-03</td>
<td>1388</td>
</tr>
<tr>
<td>Reproductive System Disease</td>
<td>4.14E-12 - 2.20E-03</td>
<td>489</td>
</tr>
</tbody>
</table>

### Physiological System Development and Function

<table>
<thead>
<tr>
<th>Name</th>
<th>p-value</th>
<th># Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological System Development and Function</td>
<td>1.67E-10 - 4.03E-03</td>
<td>1853</td>
</tr>
<tr>
<td>Hematopoiesis</td>
<td>2.04E-09 - 3.69E-03</td>
<td>424</td>
</tr>
<tr>
<td>Immune Cell Trafficking</td>
<td>5.84E-09 - 4.02E-03</td>
<td>1170</td>
</tr>
<tr>
<td>Tissue Morphology</td>
<td>9.90E-09 - 2.70E-03</td>
<td>986</td>
</tr>
<tr>
<td>Cell-mediated Immune Response</td>
<td>1.36E-06 - 2.98E-03</td>
<td>286</td>
</tr>
</tbody>
</table>
Array Design Detects AOH: Identity By Descent
Array Design Detects AOH: Uniparental Disomy
Array Design Detects AOH: Consanguinity

Sund et al., Genetics in Medicine 2013. 15(1):70-78
Pathogenic CNV In 5% Of Patients With Epilepsy

- 977 patients ICD9 code for epilepsy or seizures
- 4 excluded because CMA results were not available
- 168 excluded because no epilepsy on chart review
- 805 patients with epilepsy and chromosomal microarray (CMA)
- 482 CMA normal (60%)
- 323 at least one CNV on CMA (40%)
- Total number of CNVs 437 (1-4 per patient)
- 184 deletions (42%)
- 253 duplications (58%)

- 40 confirmed de novo (9%), 186 inherited (43%), 211 unknown inheritance (48%).
- Size range 18 kb to 142 Mb plus 3 chromosomal trisomies (X, 21, and 9)
- 147 CNVs were >500 kb in size (34%)
- At least 40/805 (5.0%) patients had CNVs explaining their epilepsy phenotype
  - 29 with syndromes including epilepsy
  - 11 with CNVs including epilepsy genes with the expected genetic pattern or deletions in known epilepsy “hotspots”

Heather et al. submitted
## Recurrent Genomic Imbalances Associated With Seizure

<table>
<thead>
<tr>
<th>Known syndrome associated with epilepsy</th>
<th># of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11 duplication syndrome</td>
<td>4</td>
</tr>
<tr>
<td>1p36 deletion syndrome</td>
<td>3</td>
</tr>
<tr>
<td>Mowat-Wilson syndrome (ZEB2 deletion, 2q22.3)</td>
<td>3</td>
</tr>
<tr>
<td>Wolf-Hirschhorn syndrome (4p16.3 deletion)</td>
<td>3</td>
</tr>
<tr>
<td>Dravet syndrome (SCN1A deletion, 2q24.3)</td>
<td>2</td>
</tr>
<tr>
<td>Williams-Beuren region reciprocal syndrome (duplication 7q11.23)</td>
<td>2</td>
</tr>
<tr>
<td>Kleefstra syndrome (9q34.3 deletion)</td>
<td>2</td>
</tr>
<tr>
<td>Angelman syndrome (15q11-q13 deletion)</td>
<td>2</td>
</tr>
<tr>
<td>Phelan-McDermid syndrome (22q13.3 deletion)</td>
<td>2</td>
</tr>
<tr>
<td>MECP2 duplication syndrome (Xq28)</td>
<td>2</td>
</tr>
<tr>
<td>1q43-q44 deletion syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Terminal 6q deletion syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Benign familial neonatal convulsions (KCNQ2 deletion, 20q13.33)</td>
<td>1</td>
</tr>
<tr>
<td>22q11.2 deletion syndrome</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total # of patients with identified genetic syndromes involving epilepsy</strong></td>
<td><strong>29/805 (3.6%)</strong></td>
</tr>
</tbody>
</table>

**Total # of patients with identified genetic syndromes involving epilepsy as a result of CMA Testing**

29/805 (3.6%)
## CNV In Single Genes Associated With Seizures

<table>
<thead>
<tr>
<th>Epilepsy gene</th>
<th>Pathogenic CNV(#)</th>
<th>Epilepsy phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLCB1 (20p12.3)</td>
<td>1 deletion, homozygous</td>
<td>Malignant migrating partial epilepsy of infancy (reported in Poduri et al. 2012)</td>
</tr>
<tr>
<td>CACNB4 (2q23.3)</td>
<td>2 deletions, heterozygous</td>
<td>Case 1 - GTCs and asymmetric tonic seizures, EEG generalized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 2 - GTCs, normal EEG</td>
</tr>
<tr>
<td>CHRNA7 (15q13.3)</td>
<td>1 deletion, heterozygous</td>
<td>Idiopathic generalized epilepsy (likely juvenile absence epilepsy) with moderate ID and hypotonia</td>
</tr>
<tr>
<td>*overlaps with hotspot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABRA1 and GABRG2 (5q34)</td>
<td>1 deletion, heterozygous</td>
<td>Mixed focal and generalized epilepsy with GTCs and focal seizures, generalized and multifocal epileptiform activity</td>
</tr>
<tr>
<td>PRRT2 (16p11.2)</td>
<td>2 deletions, heterozygous</td>
<td>Case 1 - GTCs onset within 1st years of life and controlled in setting of GDD/ID</td>
</tr>
<tr>
<td>*overlaps hotspot</td>
<td></td>
<td>Case 2 - GTCs in first year of life in setting of GDD, hypotonia, macrocephaly</td>
</tr>
<tr>
<td>Epilepsy gene</td>
<td>Possibly pathogenic CNV</td>
<td>Epilepsy phenotype</td>
</tr>
<tr>
<td>NRXN1 (2p16.3)</td>
<td>2 deletions, heterozygous</td>
<td>Case 1 - Slumping and vomiting then generalized convulsive seizures with normal EEGs, in setting of autism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case 2 - Myoclonic seizures, GTCs, and focal seizures with mixed generalized and multifocal epileptiform activity in the setting of autism</td>
</tr>
</tbody>
</table>
Detection Limitations of CMA

Microarrays will not detect

- Balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions)
- Low level mosaicism
- Point mutations
- Genomic imbalances in regions not targeted by the array design
The Past, Current, and Next-Gen Cytogenetics

Cytogenomics

Changes in copy number and regional homozygosity

Cytogenetics

Chromosomal alterations

NGS

BP resolution, genome-wide CNV, SNP and SV

CLINICAL UTILITY

RESOLUTION
The Race To Detect Cnvs By NGS

Computational tools for copy number variation (CNV) detection using next-generation sequencing data: features and perspectives

Min Zhao¹, Qiongguo Wang¹, Quan Wang¹, Peilin Jia¹, Zhongming Zhao¹,²,³*
Microarray In NGS Era: Confirming The CNV
Targeted NGS cannot detect:

• Exon-level CNVs

• Large indels

• CNV in regions not captured by exome
How can we take advantage of the maturity of microarray technology to transition into NGS based CNV detection?
Microarray In NGS Era: Filling The Gap

As companion test for single exon CNVs and large indels
Acknowledgements

Referring Health Care Providers and Their Patients

Claritas Genomics & Genetic Diagnostic Laboratories
- R&D Team
- Medical Director Team
- Clinical Team

Harvard Medical School
- Departments of Laboratory Medicine and Pathology

Agilent Technologies