Demonstrating Clinical Research Utility:

A Custom Microarray to Study Neurodevelopmental Disorders

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American College of Medical Genetics
Nashville, TN
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Boston Children's Hospital
Until every child is well™

CLARITAS GENOMICS

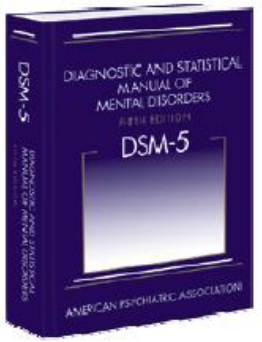
Disclosure

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)

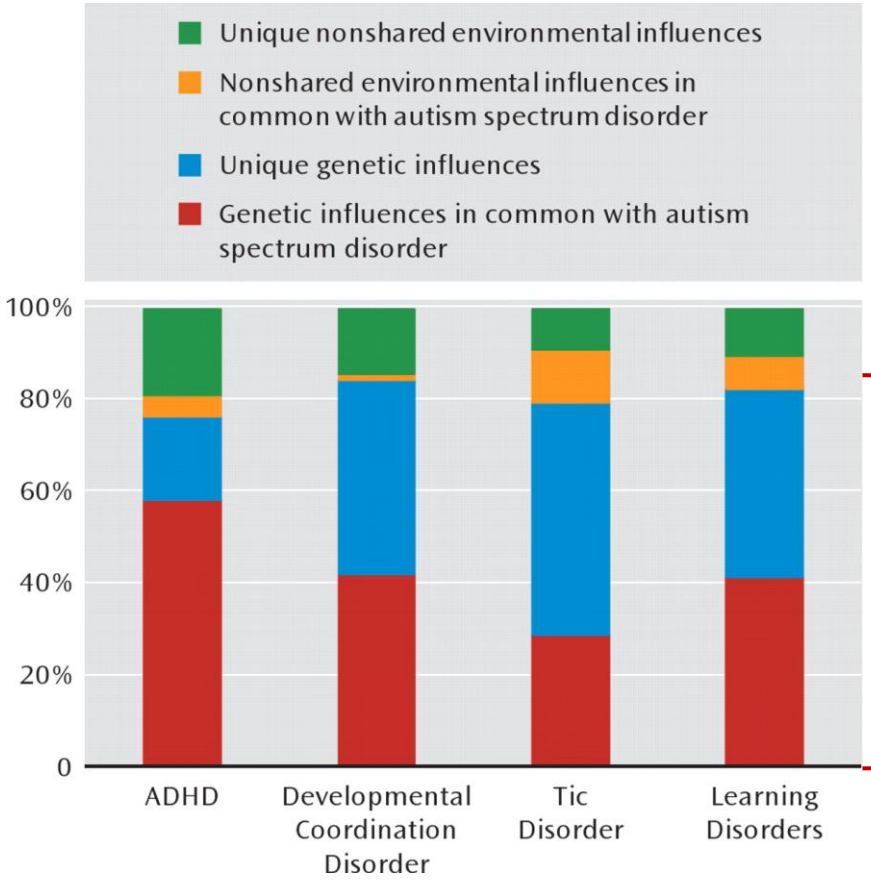


DSM-5

Neurodevelopmental Disorders

- Intellectual Disabilities
- Communication Disorders
- Autism Spectrum Disorder
- Attention-Deficit/ Hyperactivity Disorder
- Specific Learning Disorder
- Motor Disorders
- Other Neurodevelop-mental Disorders

Neurodevelopmental Disorders Are Highly Genetic



Genetic Influences

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

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

Lichtenstein et al., The Genetics of Autism Spectrum Disorders and Related Neuropsychiatric Disorders in Childhood. *Am J Psychiatry* 2010; 167:1357–1363)

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

J Med Genet 2005;**42**:699–705. doi: 10.1136/jmg.2004.029637

Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis

THE LANCET

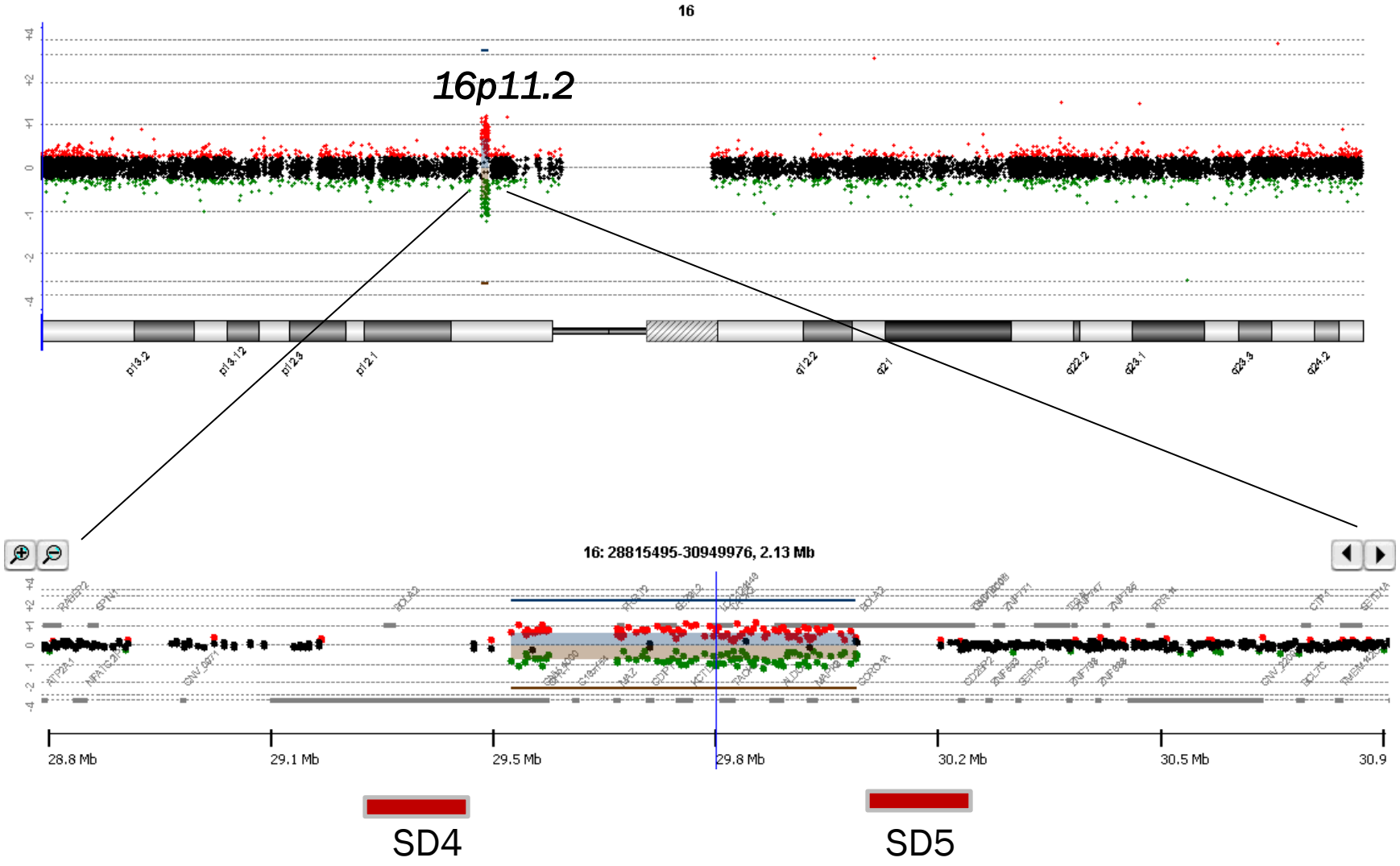
Nigel M Williams, Irina Zaharieva, Andrew Martin, Kate Langley, Kiran Mantripragada, Ragnheidur Fossdal, Hreinn Stefansson, Kari Stefansson, Pall Magnusson, Olafur O Gudmundsson, Omar Gustafsson, Peter Holmans, Michael J Owen, Michael O'Donovan, Anita Thapar

Strong Association of De Novo Copy Number Mutations with Autism

Jonathan Sebat *et al.*
Science **316**, 445 (2007);
DOI: 10.1126/science.1138659

Science
AAAS

Whole Genome Microarray (244k) Led To The Discovery Of Novel NDD Loci Such As 16p11.2



Association Between 16p11.2 Imbalances And Autism

The NEW ENGLAND JOURNAL of MEDICINE

Association between Microdeletion and Microduplication at 16p11.2 and Autism

Lauren A. Weiss, Ph.D., Yiping Shen, Ph.D., Joshua M. Korn, B.S., Dan E. Arking, Ph.D., David T. Miller, M.D., Ph.D., Ragnheidur Fossdal, B.Sc., Evald Saemundsen, B.A., Hreinn Stefansson, Ph.D., Manuel A.R. Ferreira, Ph.D., Todd Green, B.S., Orah S. Platt, M.D., Douglas M. Ruderfer, M.S., Christopher A. Walsh, M.D., Ph.D., David Altshuler, M.D., Ph.D., Aravinda Chakravarti, Ph.D., Rudolph E. Tanzi, Ph.D., Kari Stefansson, M.D., Ph.D., Susan L. Santangelo, Sc.D., James F. Gusella, Ph.D., Pamela Sklar, M.D., Ph.D., Bai-Lin Wu, M.Med., Ph.D., and Mark J. Daly, Ph.D., for the Autism Consortium.

OXFORD JOURNALS
Human Molecular Genetics

Recurrent 16p11.2 microdeletions in autism

Ravinesh A. Kumar[1], Samer KaraMohamed[1], Jyotsna Sudi[1], Donald F. Conrad[1], Camille Brune[5], Judith A. Badner[4], T. Conrad Gilliam[1], Norma J. Nowak[6], Edwin H. Cook Jr[5], William B. Dobyns[1,2,3] and Susan L. Christian[1,*]

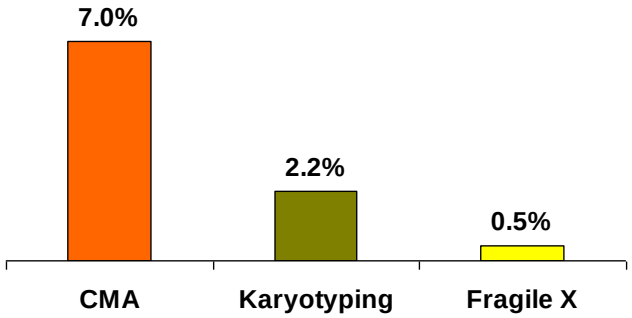
- ~0.01% in general population
- ~0.1% in individuals with psychiatric or language disorders
- ~1% in autism patients

Evidence Suggests Microarray As First Line Test For ASD

PEDIATRICS
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Clinical Genetic Testing for Patients With Autism Spectrum Disorders

AUTHORS: Yiping Shen, PhD,[a,b,c,d] Kira A. Dies, ScM,[a,c] Ingrid A. Holm, MD, MPH,[a,c,e,f] Carolyn Bridgemohan, MD,[a,c,g] Magdi M. Sobeih, MD, PhD,[a,c,h] Elizabeth B. Caronna, MD,[a,i] Karen J. Miller, MD,[a,j] Jean A. Frazier, MD,[a,k,l] Iris Silverstein, MD,[a,m] Jonathan Picker, MBChB, PhD,[a,c,n] Laura Weissman, MD,[a,c,g] Peter Raffalli, MD,[a,c,h] Shafali Jeste, MD,[a,c,h] Laurie A. Demmer, MD,[a,j] Heather K. Peters, MS,[a,e] Stephanie J. Brewster, MS,[a,e] Sara J. Kowalczyk, MA, MPH,[a,i] Beth Rosen-Sheidley, MS,[a,j] Caroline McGowan, MS,[a,n] Andrew W. Duda, III, MS,[a,m] Sharyn A. Lincoln, MS,[a,n] Kathryn R. Lowe, MS,[a,e] Alison Schonwald, MD,[a,c,g] Michael Robbins, MD,[a,c,h] Fuki Hisama, MD,[a,c,n] Robert Wolff, MD,[a,c,h] Ronald Becker, MD,[a,c,g] Ramzi Nasir, MD, MPH,[a,c,g] David K. Urion, MD,[a,c,h] Jeff M. Milunsky, MD,[a,i,o] Leonard Rappaport, MD,[a,c,g] James F. Gusella, PhD,[a,c,d] Christopher A. Walsh, MD, PhD,[a,c,n] Bai-Lin Wu, PhD, MMed,[a,b,c,p] and David T. Miller, MD, PhD[a,b,c,n], on behalf of the Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration



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.

ISCA Consortium Consensus Statement

AJHG The American Journal of Human Genetics ARTICLE

Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M. Ostell,[8] Carla Rosenberg,[20] Stephen W. Scherer,[21] Nancy B. Spinner,[17] Dimitri J. Stavropoulos,[22] James H. Tepperberg,[23] Erik C. Thorland,[24] Joris R. Vermeesch,[25] Darrel J. Waggoner,[26] Michael S. Watson,[27] Christa Lese Martin,[2] and David H. Ledbetter[2,*]

"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."

ACMG Practice Guideline

Genetics in Medicine
The Journal of the American College of Medical Genetics

Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities

Melanie Manning, MD, MS FACMG[1] and Louanne Hudgins, MD, FACMG[1]

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.

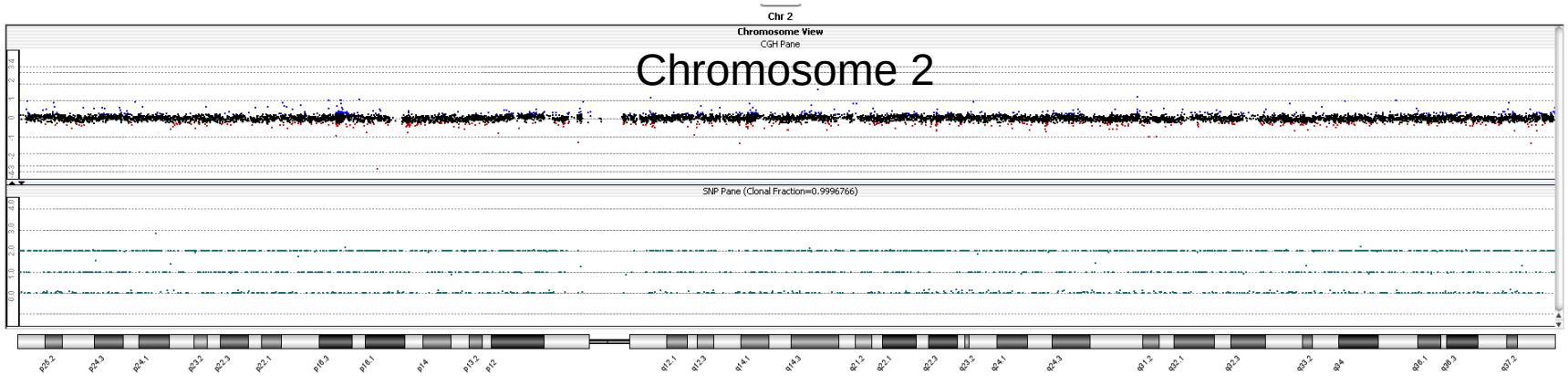
Rationale For Redesigning The Array

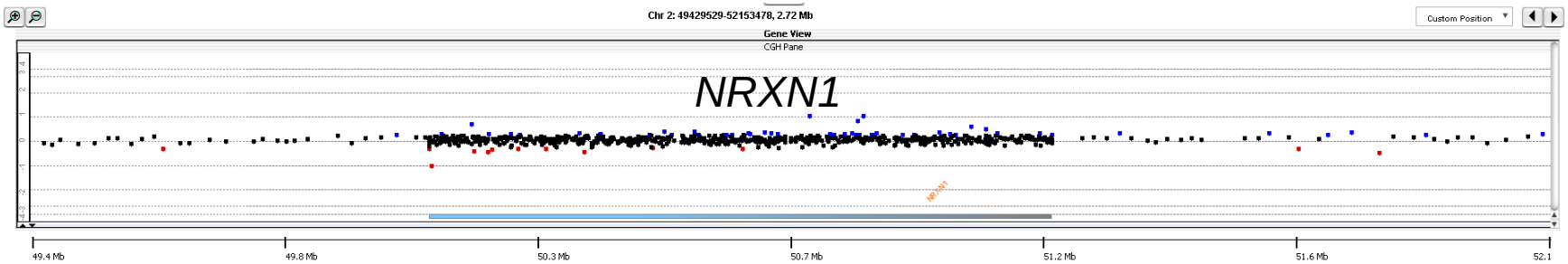
- Clinical relevance differs in different regions of the human genome
- Both large genomic imbalances and small intragenic CNVs contribute to NDD
- The vast majority of CNVs are population polymorphisms and clinically benign
- Long contiguous stretches of homozygosity (LCSH) may be clinically significant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

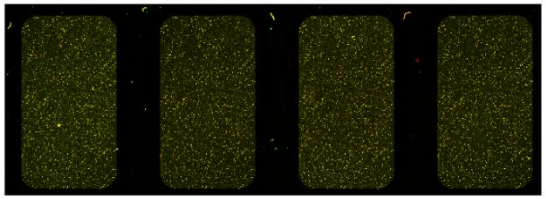
The Basis For Our Custom Design

- >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







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.

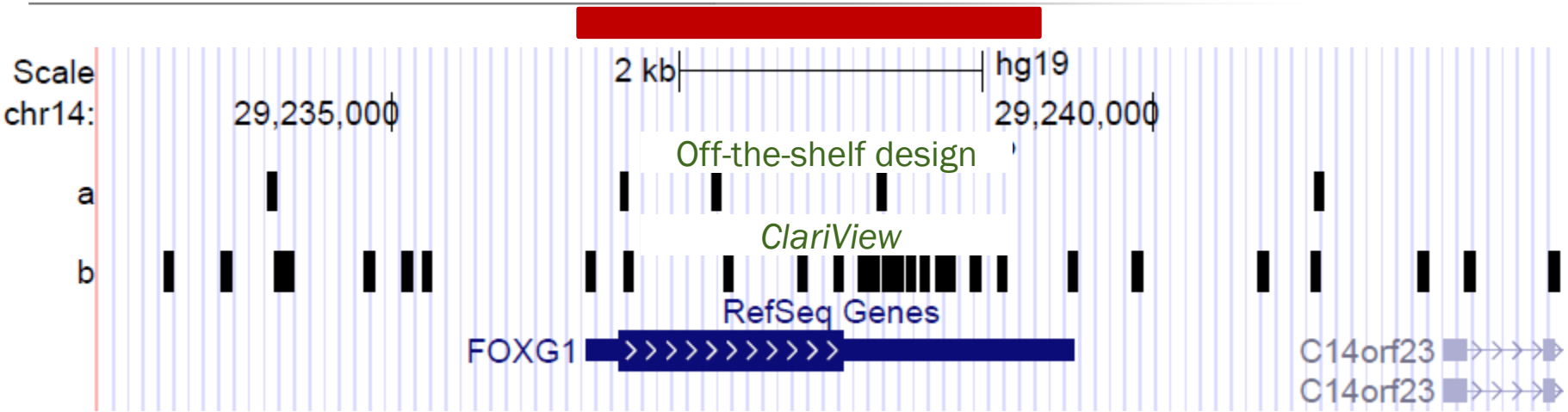
2. ~1000 NDD genes with enhanced coverage.

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

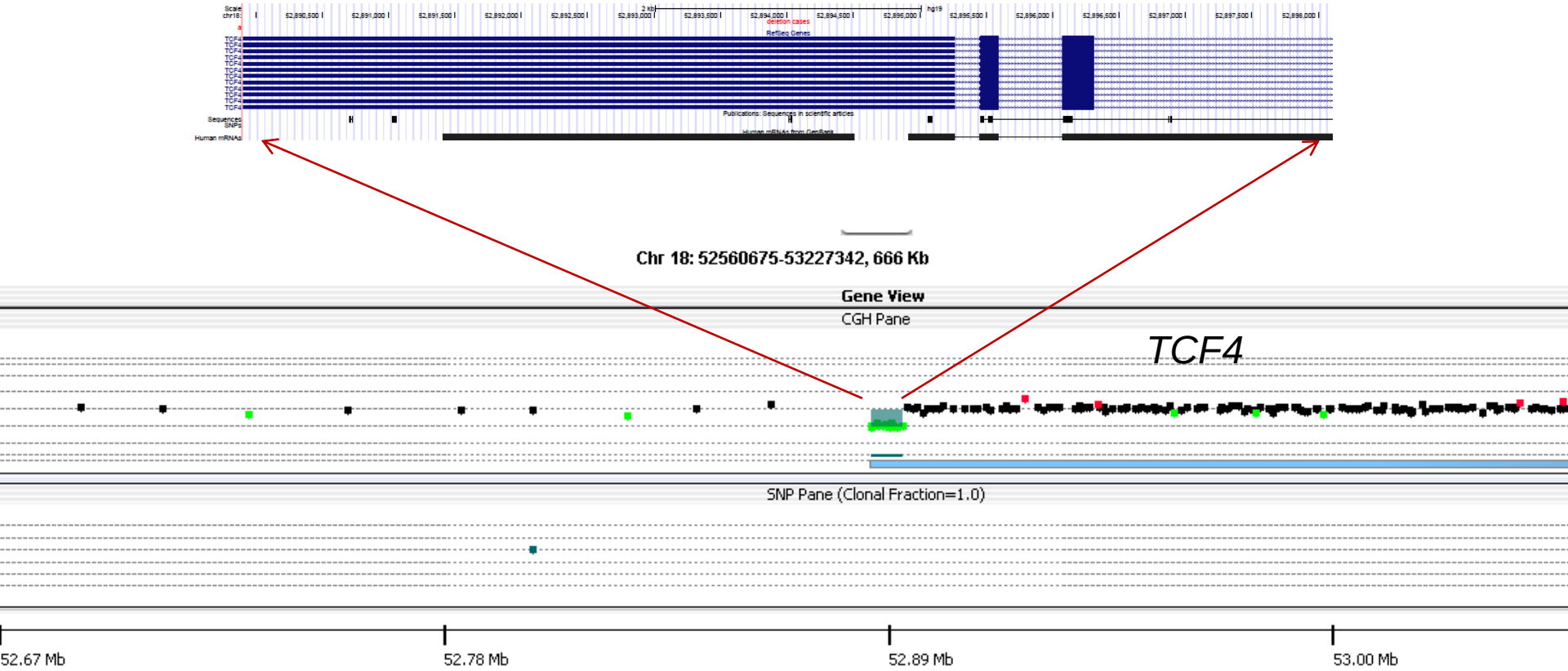
1K Resolution In Key Clinically-Relevant Regions



CHIP	Total probe #	Interval (kb)	Probe density	Detection sensitivity (kb)
Claritas	16	3.2	5	1
Off-the-shelf	3	3.2	0.94	>5.3

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 *YWHAE* gene.

Genes Covered By > 5 Probes

Diseases and Disorders

Name	p-value	# Molecules
Cancer	2.02E-100 - 3.43E-08	2100
Gastrointestinal Disease	9.23E-90 - 2.77E-08	1474
Developmental Disorder	1.37E-47 - 3.65E-08	877
Neurological Disease	1.37E-47 - 3.65E-08	1053
Cardiovascular Disease	5.77E-45 - 2.04E-08	515

Physiological System Development and Function

Name	p-value	# Molecules
Organismal Survival	1.68E-67 - 7.05E-25	878
Nervous System Development and Function	3.50E-52 - 3.73E-08	908
Embryonic Development	4.49E-43 - 4.13E-08	834
Organ Development	4.49E-43 - 3.89E-08	674
Organismal Development	4.49E-43 - 3.89E-08	1006

Little Or No Coverage In Common Polymorphic CNVs

Probe distribution track

Copy number polymorphism track

Genes Covered by < 5 Probes

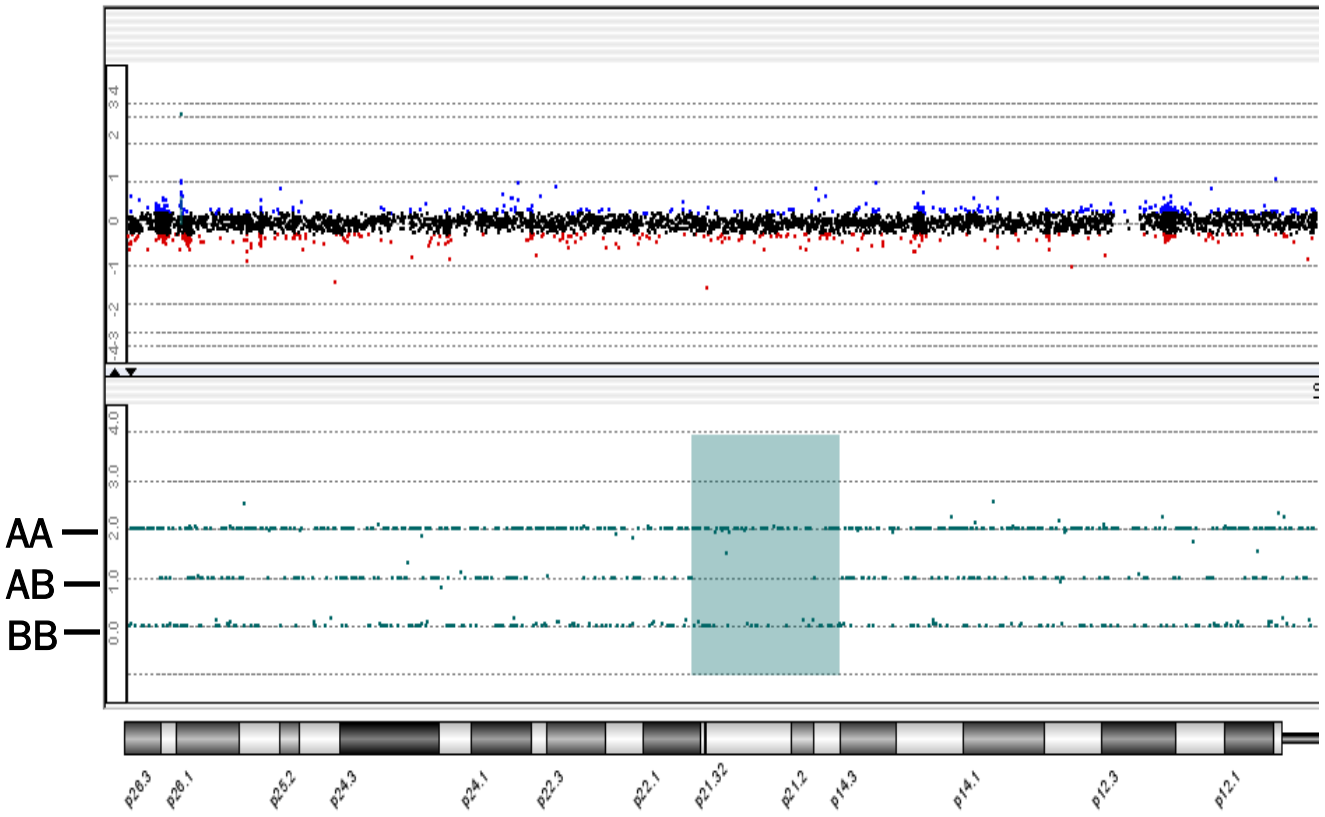
Diseases and Disorders

Name	p-value	# Molecules
Cancer	2.50E-47 - 4.02E-03	6764
Infectious Disease	1.76E-24 - 3.28E-03	1983
Dermatological Diseases and Conditions	2.60E-14 - 3.60E-03	935
Immunological Disease	3.86E-13 - 2.72E-03	1388
Reproductive System Disease	4.14E-12 - 2.20E-03	489

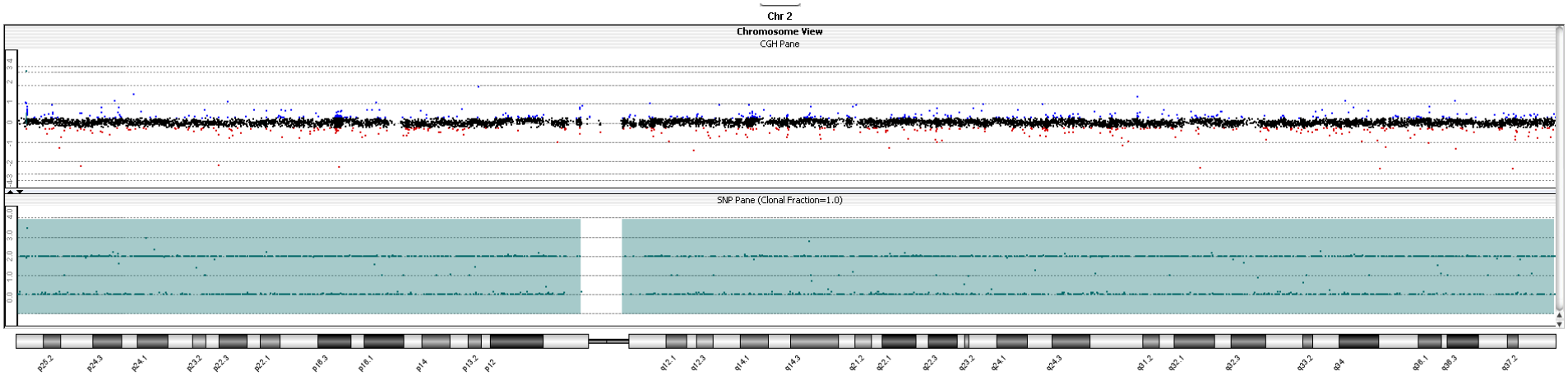
Physiological System Development and Function

Name	p-value	# Molecules
Hematological System Development and Function	1.67E-10 - 4.03E-03	1853
Hematopoiesis	2.04E-09 - 3.69E-03	424
Immune Cell Trafficking	5.84E-09 - 4.02E-03	1170
Tissue Morphology	9.90E-09 - 2.70E-03	986
Cell-mediated Immune Response	1.36E-06 - 2.98E-03	286

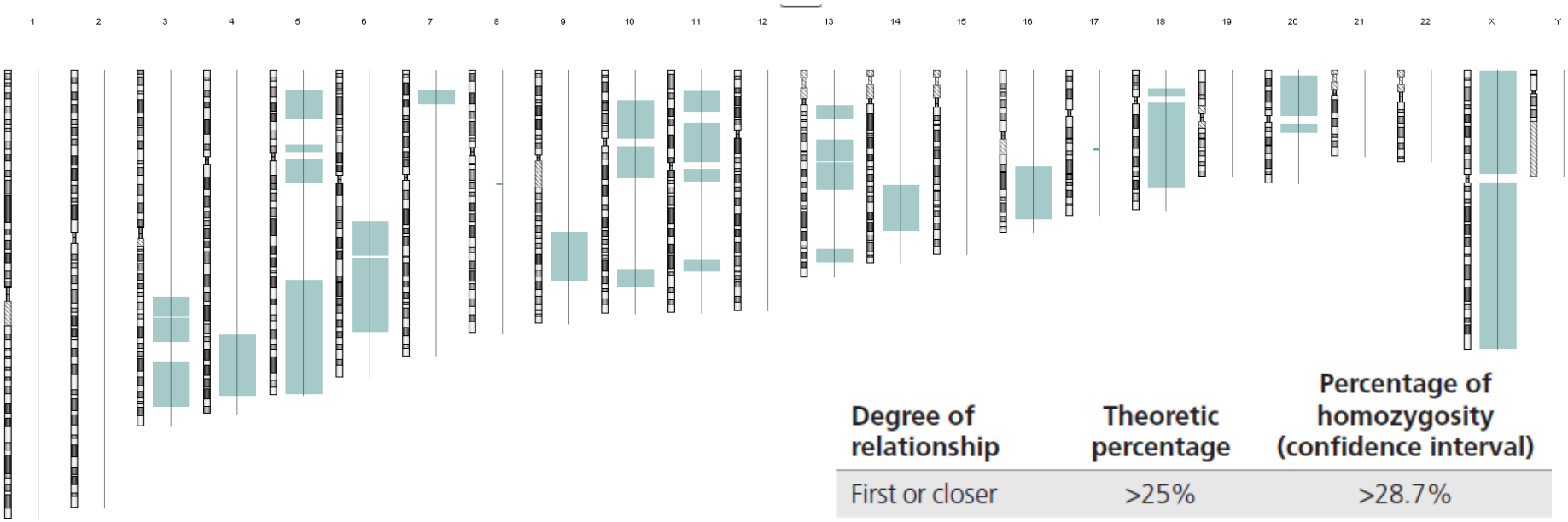
Array Design Detects AOH: Identity By Descent



Array Design Detects AOH: Uniparental Disomy



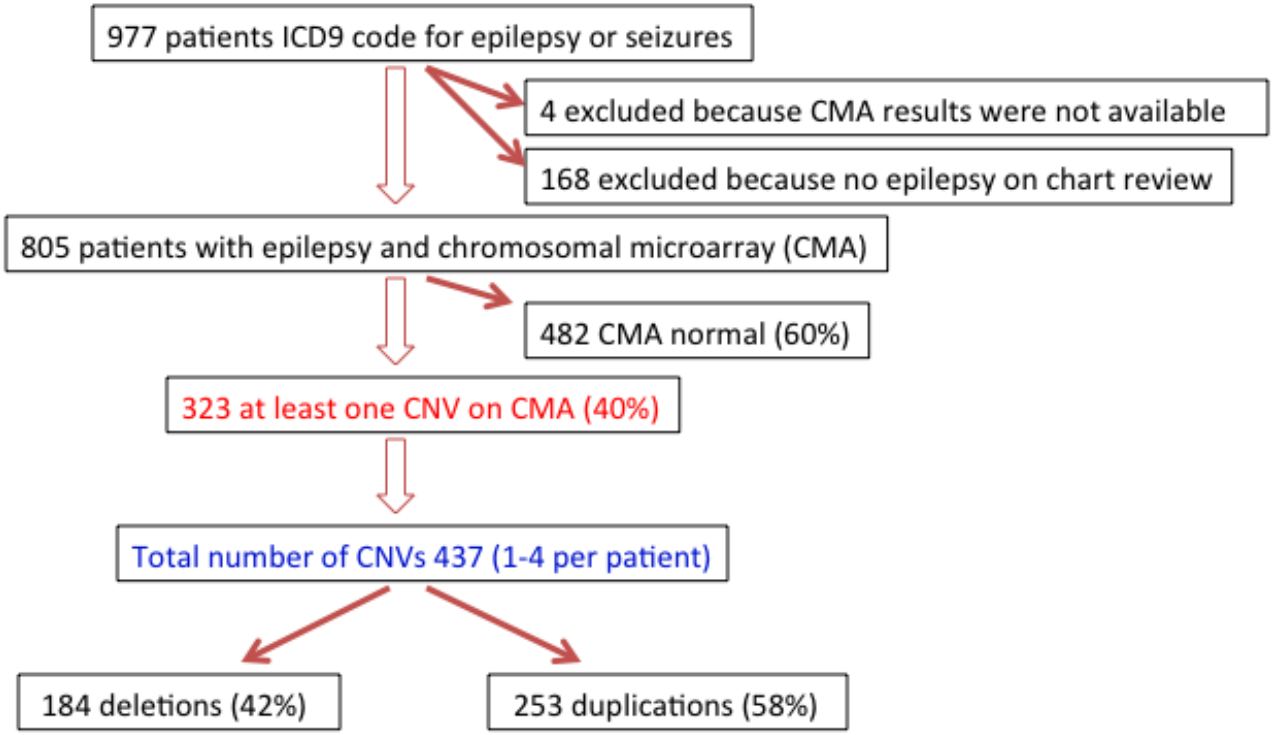
Array Design Detects AOH: Consanguinity



Degree of relationship	Theoretic percentage	Percentage of homozygosity (confidence interval)
First or closer	>25%	>28.7%
First	25%	21.3–28.7%
First or second		15.3–21.3%
Second	12.5%	9.7–15.3%
Second or third		8.3–9.7%
Third	6.25%	4.6–8.3%
Third or fourth		4.2–4.6%
Fourth	3.125%	2.6–4.2%
Fourth or fifth		1.6–2.6%
Fifth	1.5625%	0.5–1.6%

Sund et al., *Genetics in Medicine* 2013. 15(1):70-78

Pathogenic CNV In 5% Of Patients With Epilepsy



- 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

Known syndrome associated with epilepsy	# of cases
22q11 duplication syndrome	4
1p36 deletion syndrome	3
Mowat-Wilson syndrome (ZEB2 deletion, 2q22.3)	3
Wolf-Hirschhorn syndrome (4p16.3 deletion)	3
Dravet syndrome (SCN1A deletion, 2q24.3)	2
Williams-Beuren region reciprocal syndrome (duplication 7q11.23)	2
Kleefstra syndrome (9q34.3 deletion)	2
Angelman syndrome (15q11-q13 deletion)	2
Phelan-McDermid syndrome (22q13.3 deletion)	2
MECP2 duplication syndrome (Xq28)	2
1q43-q44 deletion syndrome	1
Terminal 6q deletion syndrome	1
Benign familial neonatal convulsions (KCNQ2 deletion, 20q13.33)	1
22q11.2 deletion syndrome	1
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

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

Detection Limitations of CMA

Microarrays will not detect

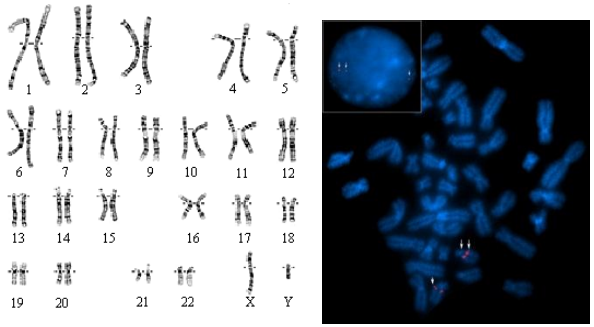
- Balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions)
- Low level mosaicism
- Point mutations
- Genomic imbalances in regions not targeted by the array design

The Past, Current, and Next-Gen Cytogenetics

CLINICAL UTILITY

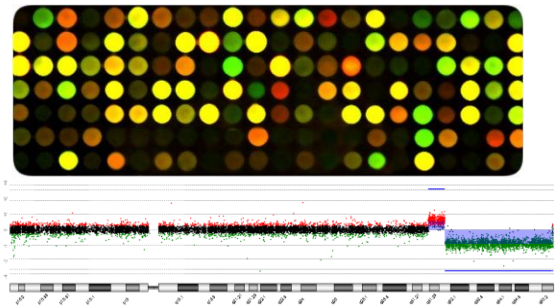
RESOLUTION

Cytogenetics



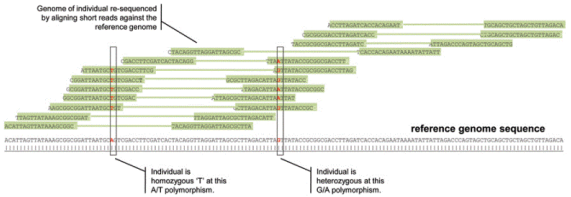
Chromosomal alterations

Cytogenomics



Changes in copy number and regional homozygosity

NGS



BP resolution, genome-wide CNV, SNP and SV

The Race To Detect Cnvs By NGS

Zhao et al. BMC Bioinformatics 2013, 14(Suppl 11):S1
http://www.biomedcentral.com/1471-2105/14/S11/S1

BMC Bioinformatics

RESEARCH Open Access

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

Min Zhao[1], Qingguo Wang[1], Quan Wang[1], Peilin Jia[1], Zhongming Zhao[1,2,3*]

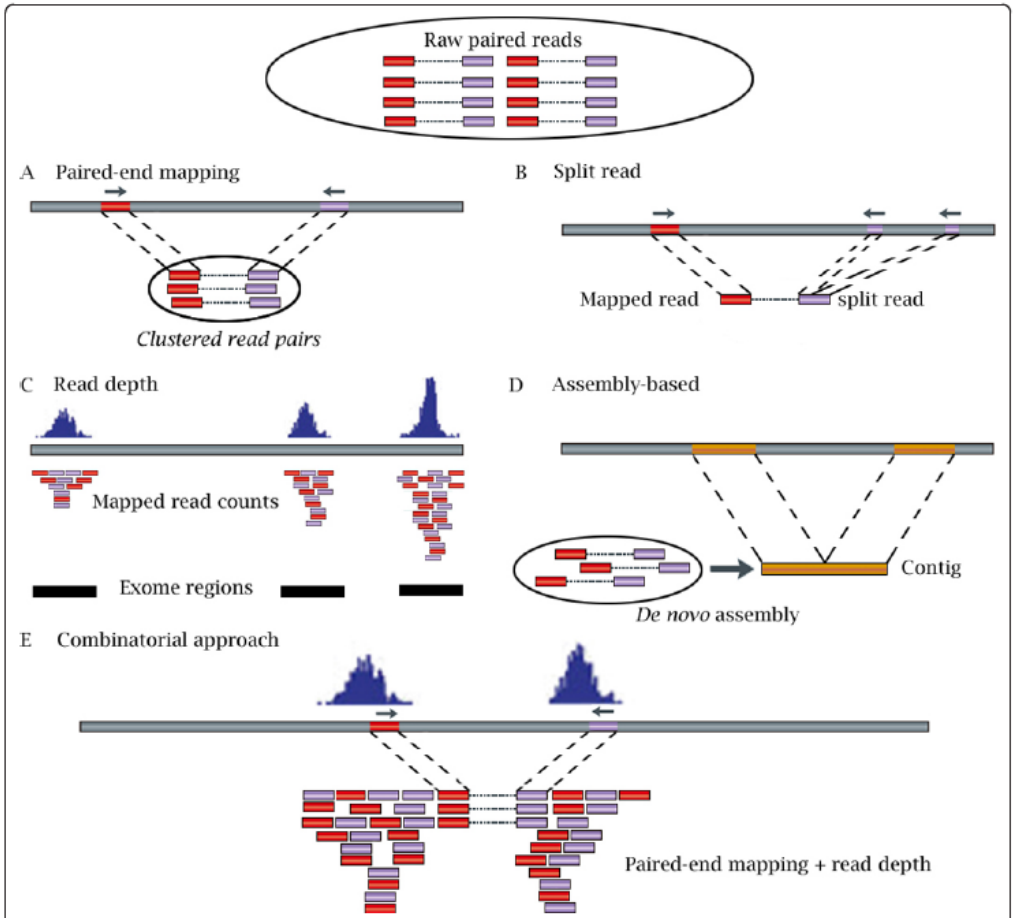
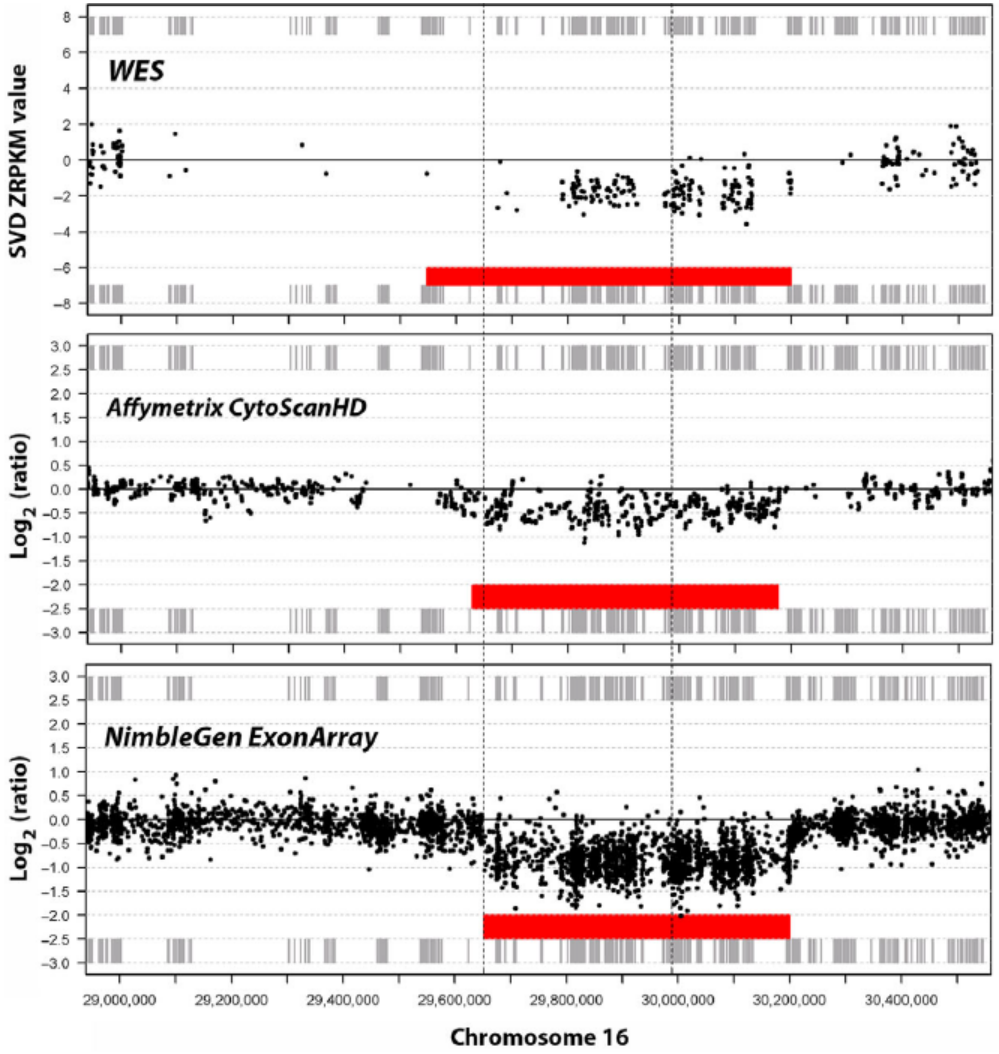
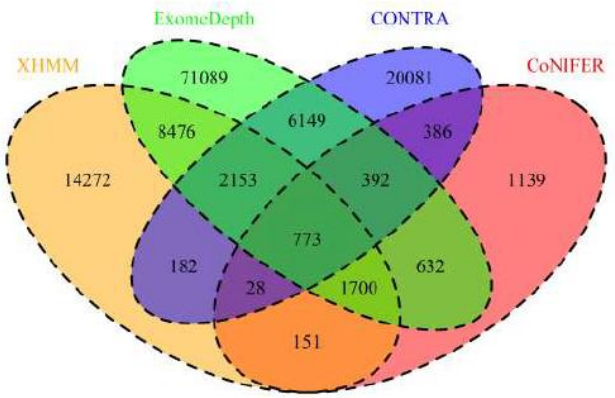


Figure 1 Five approaches to detect CNVs from NGS short reads. A. Paired-end mapping (PEM) strategy detects CNVs through discordantly mapped reads. A discordant mapping is produced if the distance between two ends of a read pair is significantly different from the average insert size. B. Split read (SR)-based methods use incompletely mapped read from each read pair to identify small CNVs. C. Read depth (RD)-based approach detects CNV by counting the number of reads mapped to each genomic region. In the figure, reads are mapped to three exome regions. D. Assembly (AS)-based approach detects CNVs by mapping contigs to the reference genome. E. Combinatorial approach combines RD and PEM information to detect CNVs.

Microarray In NGS Era: Confirming The CNV





An Evaluation of Copy Number Variation Detection Tools from Whole-Exome Sequencing Data

Renjie Tan[1,2], Yadong Wang[1*], Sarah E. Kleinstein[2,3], Yongzhuang Liu[1,2], Xiaolin Zhu[2], Hongzhe Guo[1,2], Qinghua Jiang[1], Andrew S. Allen[2,4], Mingfu Zhu[2,5*]

METHODS

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Detection of Clinically Relevant Copy Number Variants with Whole-Exome Sequencing

Joep de Ligt,[1†] Philip M. Boone,[2†] Rolph Pfundt,[1] Lisenka E.L.M. Vissers,[1] Todd Richmond,[3] Joel Geoghegan,[3] Kathleen O'Moore,[3] Nicole de Leeuw,[1] Christine Shaw,[2,3] Han G. Brunner,[1] James R. Lupski,[2,4,5] Joris A. Veltman,[1] and Jayne Y. Hehir-Kwa[1*]

Targeted NGS cannot detect:

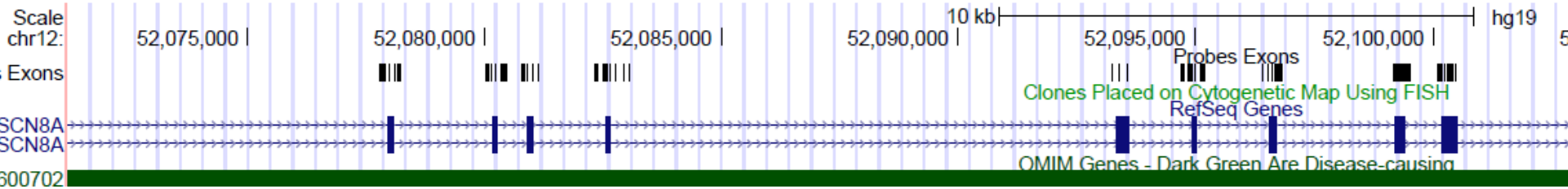
- Exon-level CNVs
- Large indels
- CNV in regions not captured by exome

NGS for CNV detection-step into the future

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



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- R&D Team
- Medical Director Team
- Clinical Team

Harvard Medical School

- Departments of Laboratory Medicine and Pathology

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