ISCA: International Standard Cytogenomic Array (ISCA) Consortium and Database

July 8, 2009

David H. Ledbetter, Ph.D.

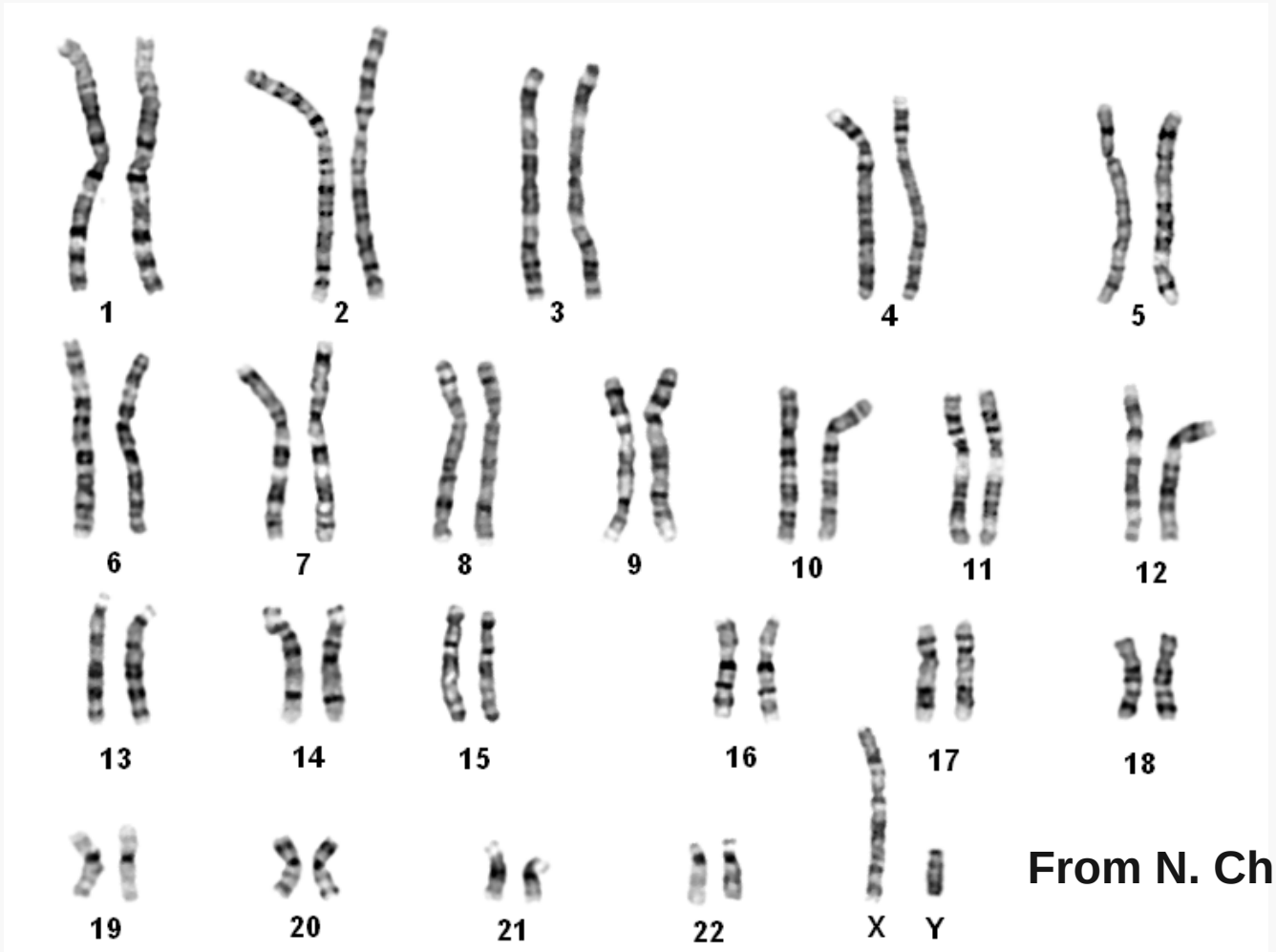
david.ledbetter@emory.edu

1981

“Cytogenetics will become extinct within the next 5 years.”

C. Thomas Caskey, M.D., Chair
Department of Human Genetics
Baylor College of Medicine

Cytogenetics:
The FIRST whole genome technology!



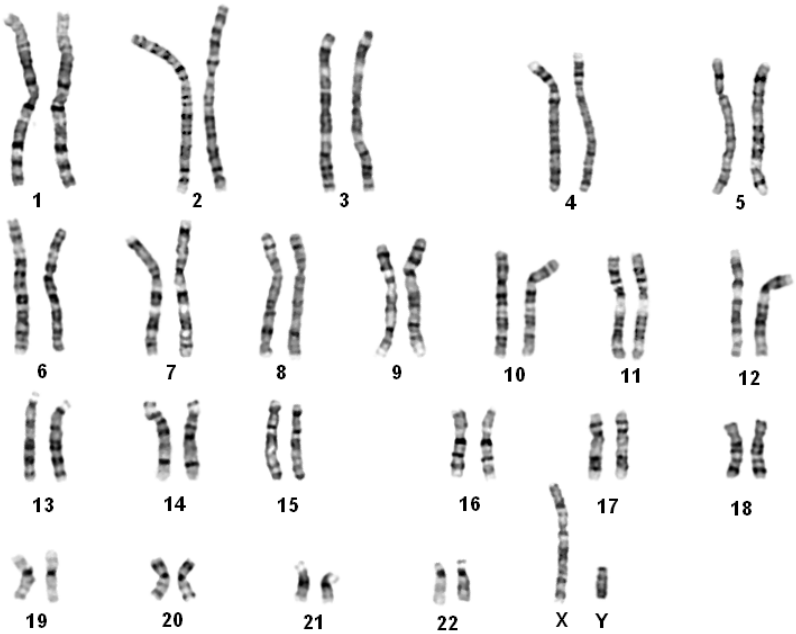
From N. Chia

Requires 500-600 evenly spaced DNA probes to match the power of the karyotype.

Key Features of G-banded Karyotype

- First whole-genome technology to detect clinically significant genomic imbalances (deletions, duplications)

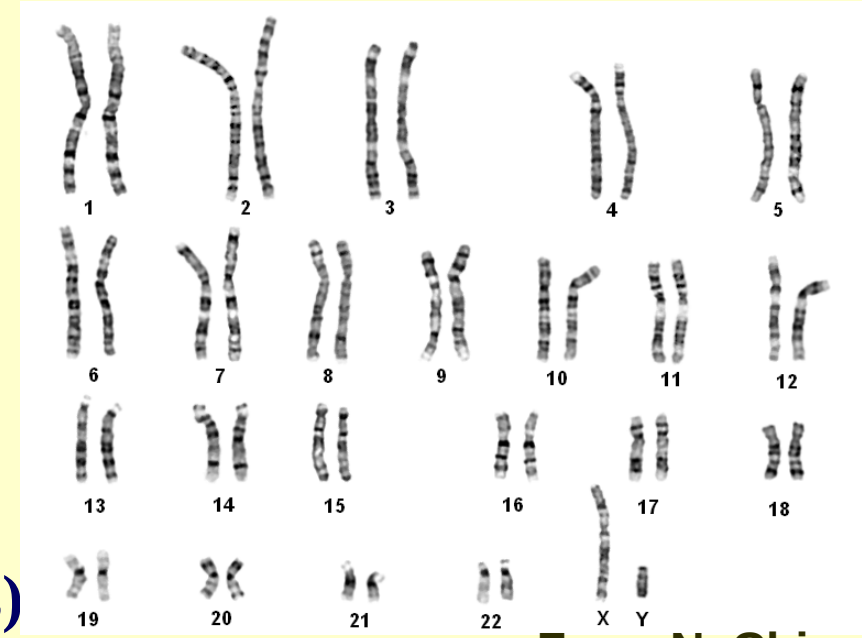
- Benign polymorphisms (CNVs) identified by empiric experience over a number of years



From N. Chia

Gene dosage lessons from 50 years of cytogenetics experience

- Monosomy and deletions cause more severe phenotypic consequences than trisomy and duplications
  - No viable autosomal monosomies (only 45,X)



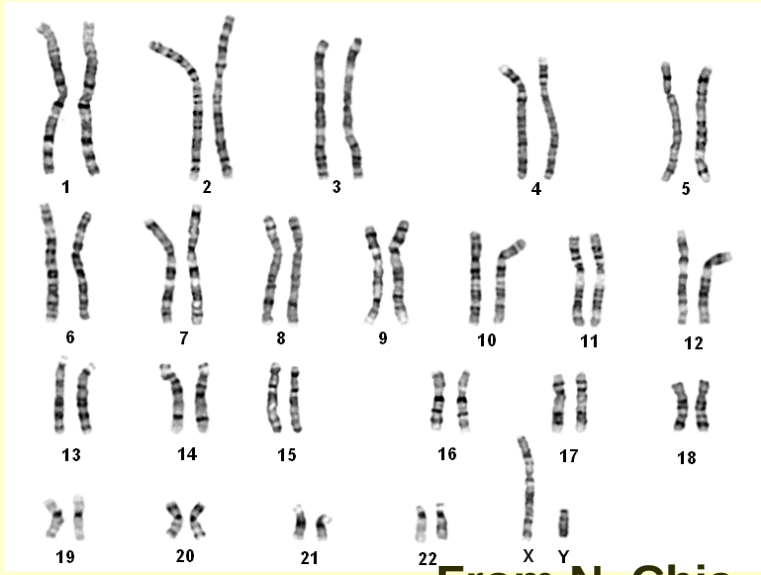
From N. Chia

- Larger imbalances (more genes) more severe phenotype than smaller imbalances

- Imbalance of G-negative bands (gene-rich) more severe than G-positive bands (gene-poor)

Gene dosage lessons from 50 years of cytogenetics experience

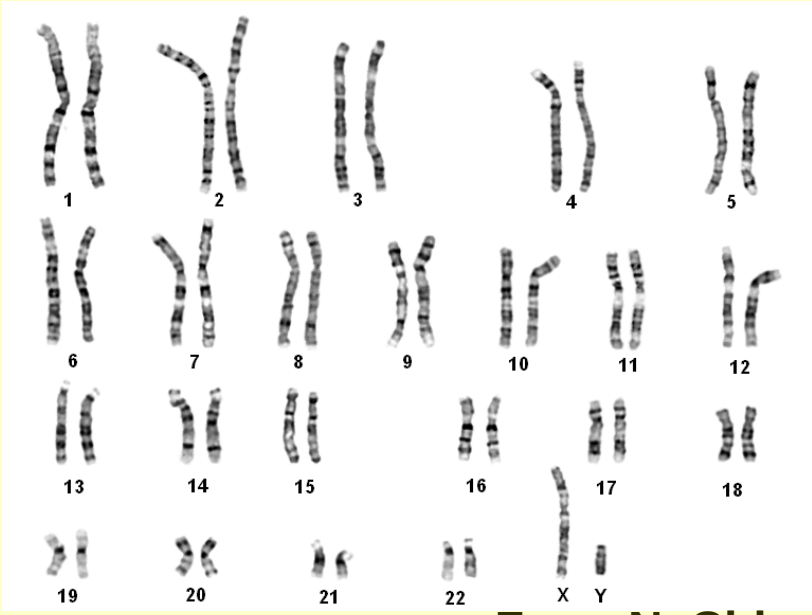
- Not all genes are dosage-sensitive
  - Down syndrome "critical region"
- phenotype in microdeletion syndromes attributed to 1 or few genes (*UBE3A* -> Angelman syndrome)



From N. Chia

Key Features of G-banded Karyotype

- **Clinical significance of imbalance in proband sometimes requires parental studies to determine if pathogenic or benign (de novo taken as evidence likely pathogenic)**

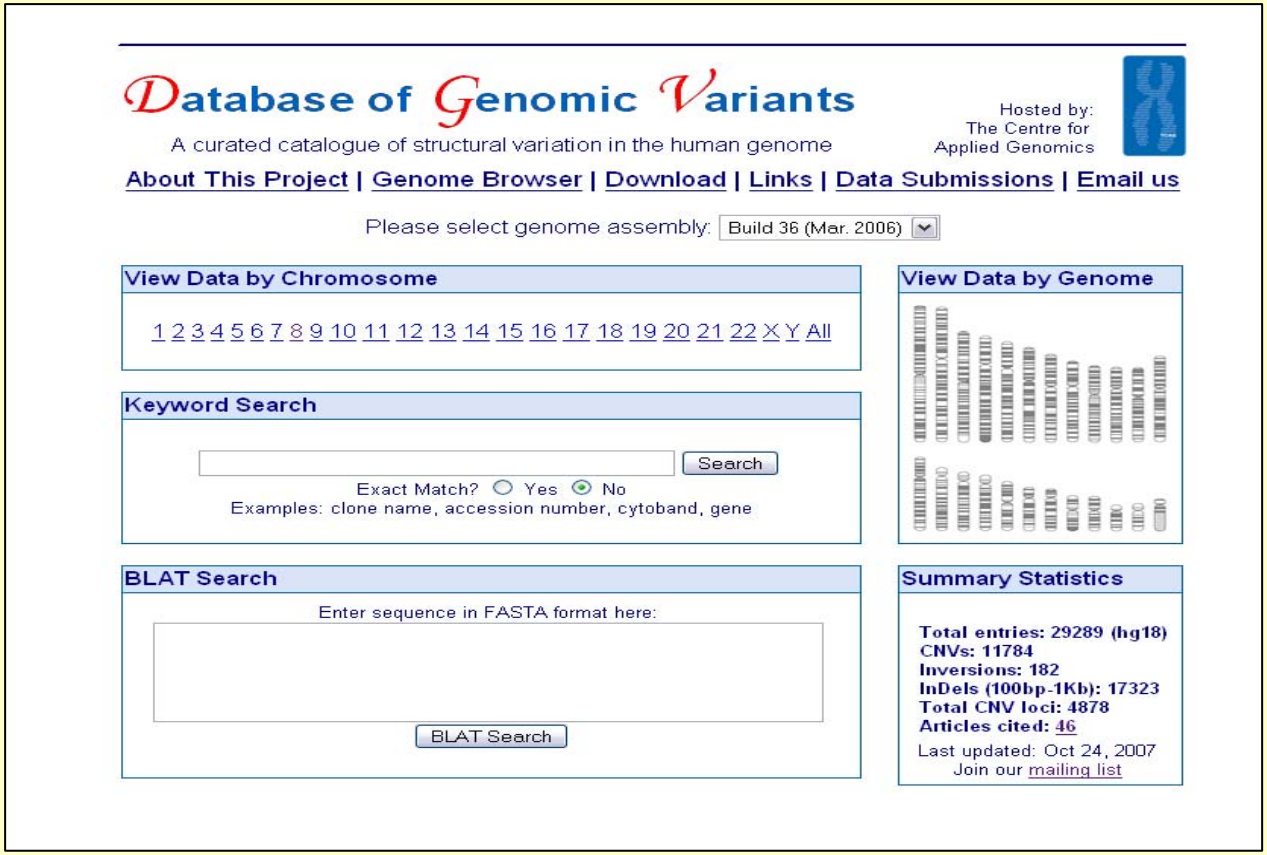


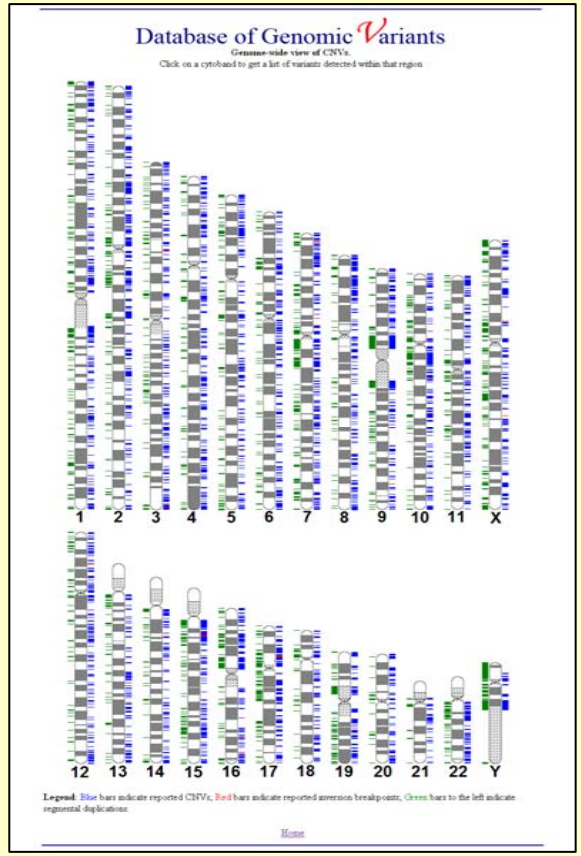
From N. Chia

But, limited resolution (5-10 Mb), variable quality and subjective interpretation

Lesson 1: The "Gold Standard" karyotype has become tarnished

How much structural variation is there in humans?
-individual and population (*note vast majority is CNV)





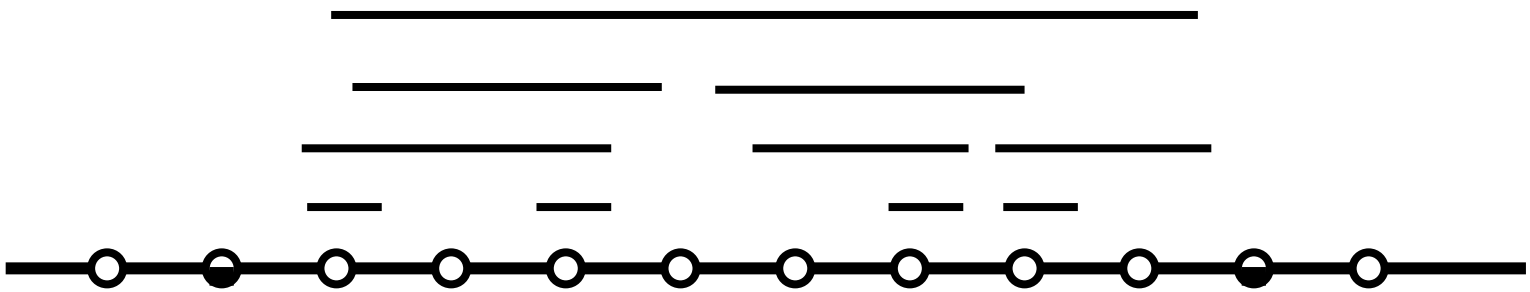
http://projects.tcag.ca/variation/

Iafrate et al, *Nature Genetics 2004*

Gene Dosage Map and CNVs

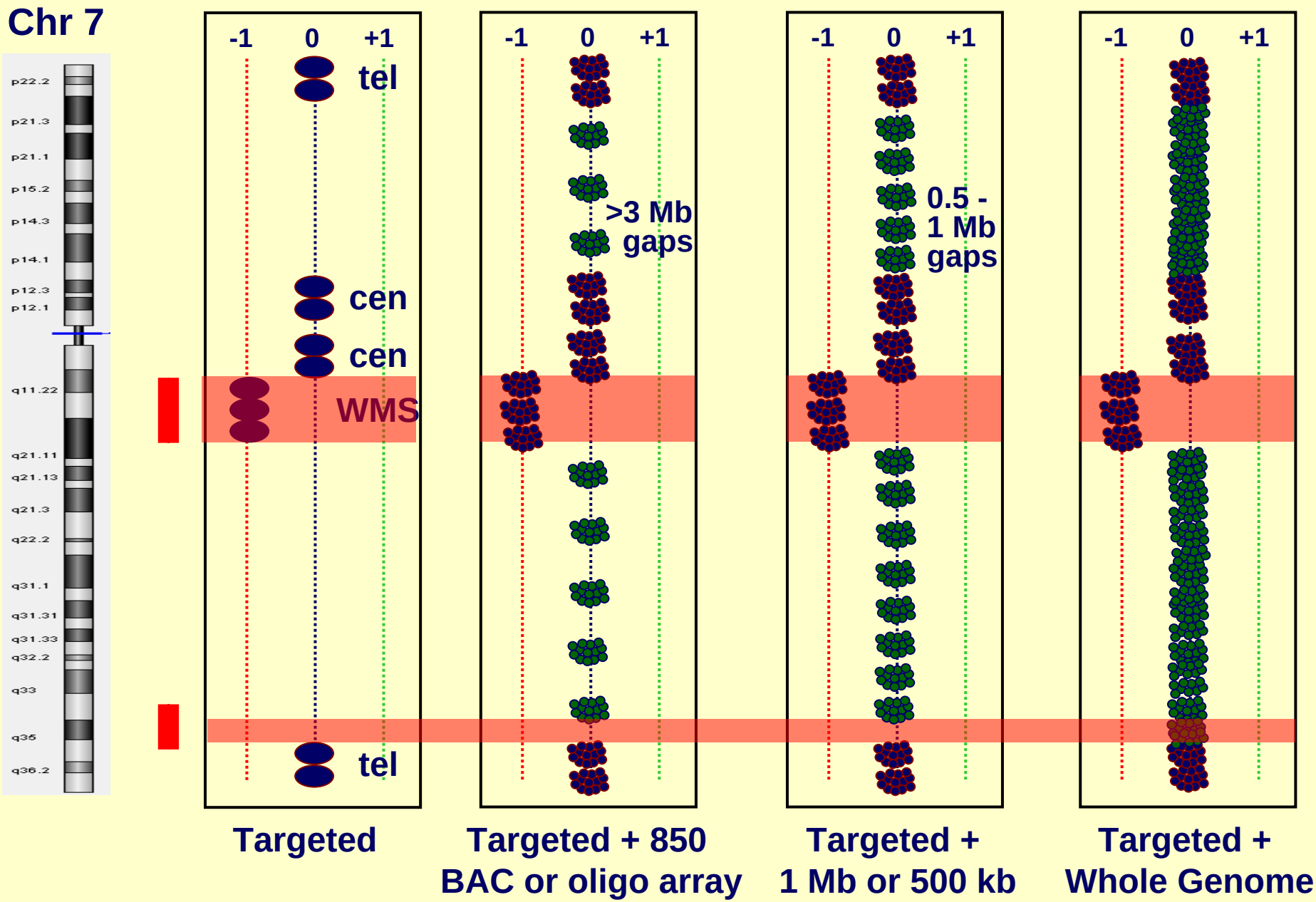
How many genes in the genome are dosage sensitive? (haploinsufficiency or triplosensitive)

- **Probably a minority (? 5-10%).**
- **Many genes are not dosage sensitive**
  - **heterozygous carriers for autosomal recessive disorders**



? 10 CNVs or 1 dosage insensitive region with an infinite # of possible CNVs

Evolution of Array Designs



Targeted + Whole Genome Arrays

Genet Med 2008:10(6):415–429.

article

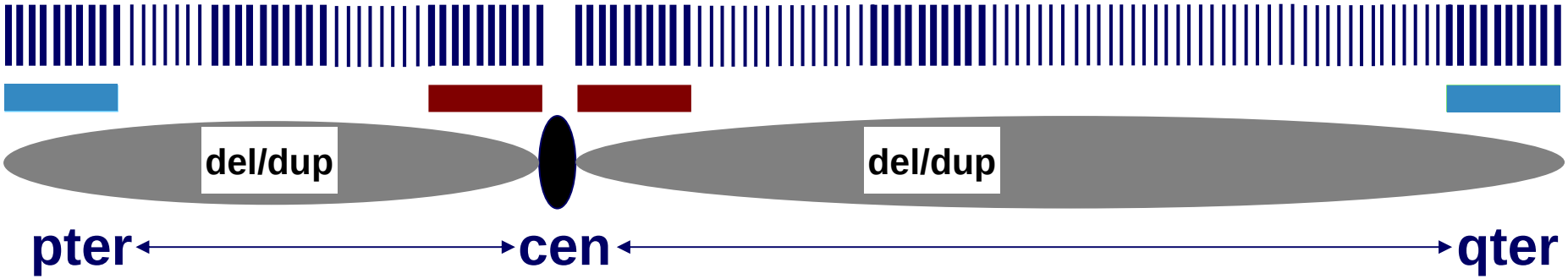
Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray

Erin L. Baldwin, PhD, Ji-Yun Lee, PhD, Douglas M. Blake, BS, Brian P. Bunke, BS, Chad R. Alexander, BS, Amy L. Kogan, BS, David H. Ledbetter, PhD, and Christa L. Martin, PhD

Oligonucleotide microarray (60mers)

Custom-designed 4x44k format - Agilent

Custom Array Design by Clinical Cytogeneticists & Clinical Geneticists



Resolution

- Telomere FISH clone
- Unique centromere FISH clone
- Known clinically relevant regions

~50 kb

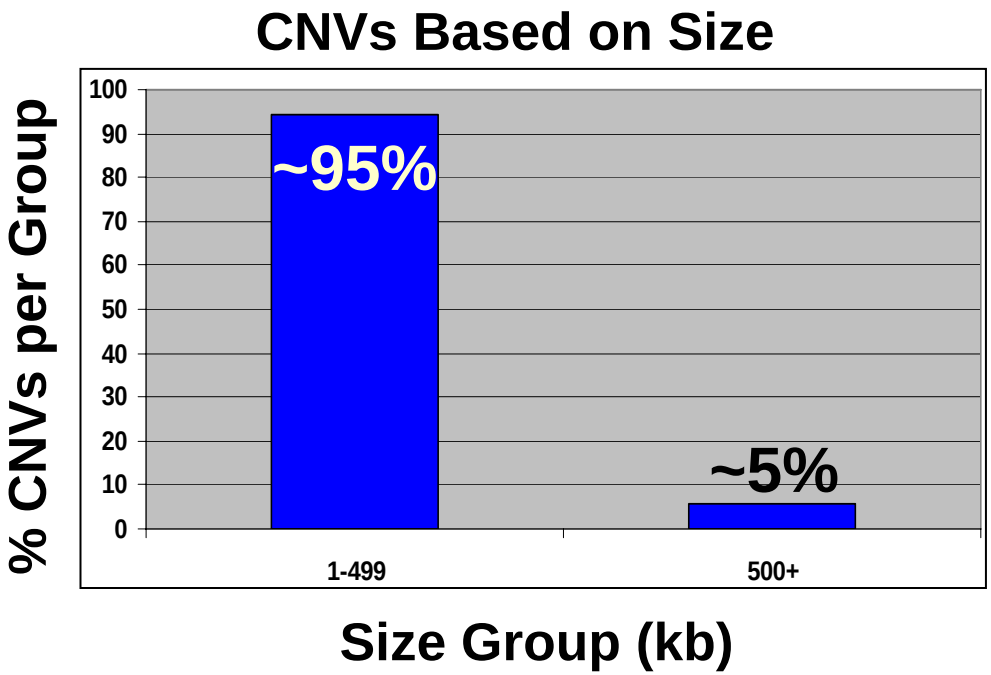
- 75 kb interval backbone

~250 (500) kb

Baldwin et al., 2008

Why Use a 500kb Triage?

- Database of Genomic Variants (Oct. 2006) (http://projects.tcag.ca/variation/



- Redon et al. (Nature 2006) – 81 kb median with 500K array
- Lee et al. (unpublshed) – 2.7 kb median with 4.2 M array

Pathogenic vs. Benign Copy Number Changes

1. **Region of known clinical significance:**
   - **known del/dup or Mendelian disorders**
   - **known benign CNC**
   - **comparison with other cases in literature, databases**

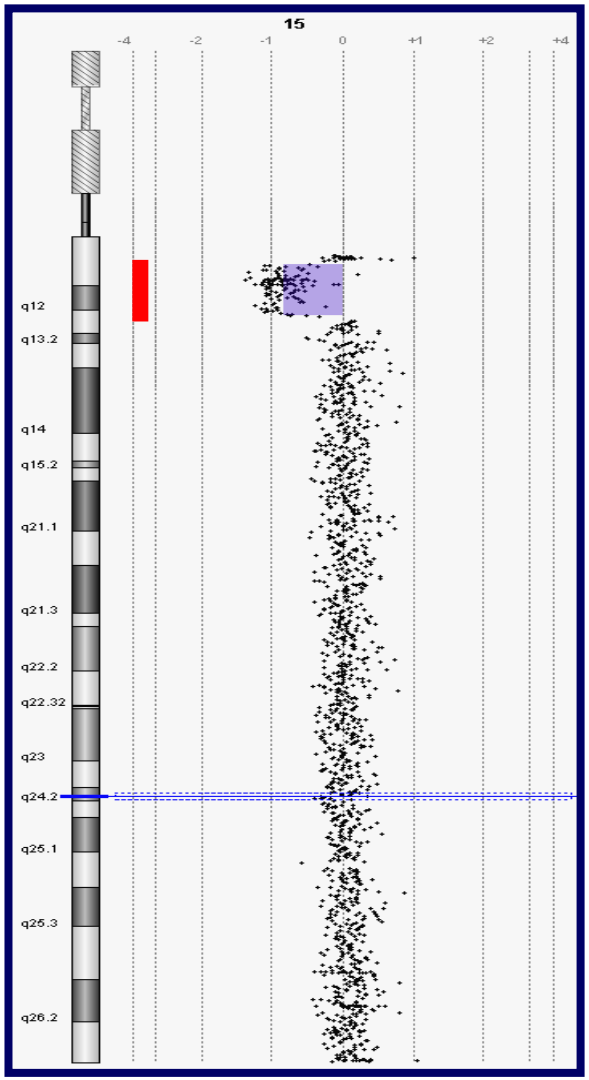
2. **Gene Content**
   - **correlates with size and location**

   (G- bands gene-rich; G+ gene-poor)

3. **Inherited or *de novo* (need parental samples in <5% of cases)**

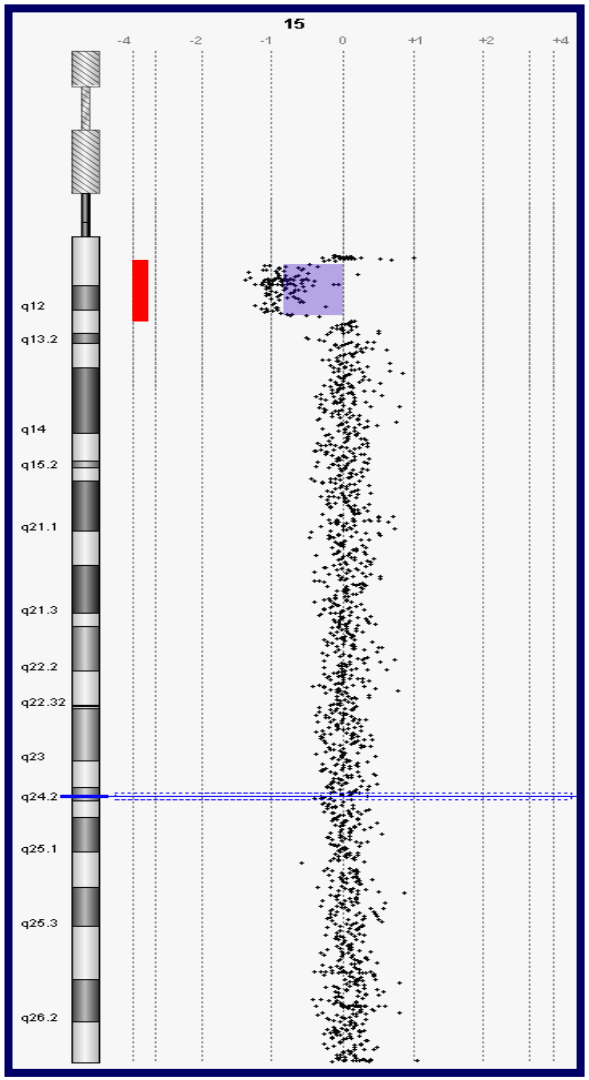
Targeted Coverage: PWS/AS Region

PWS/AS deletion

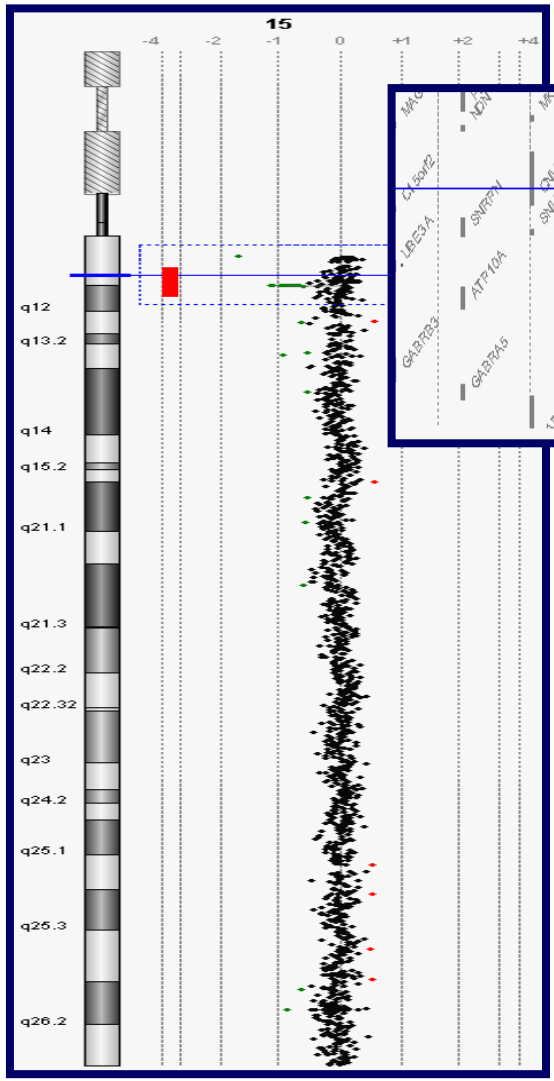


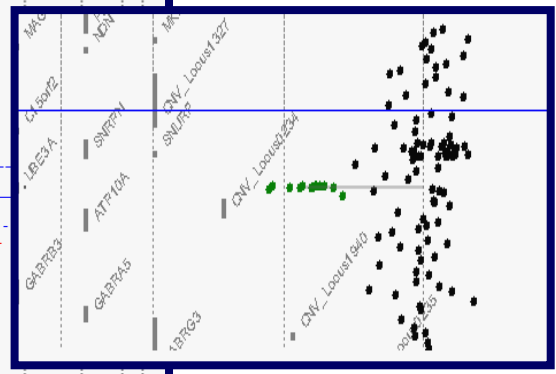
Targeted Coverage: PWS/AS Region

PWS/AS deletion



Atypical deletion



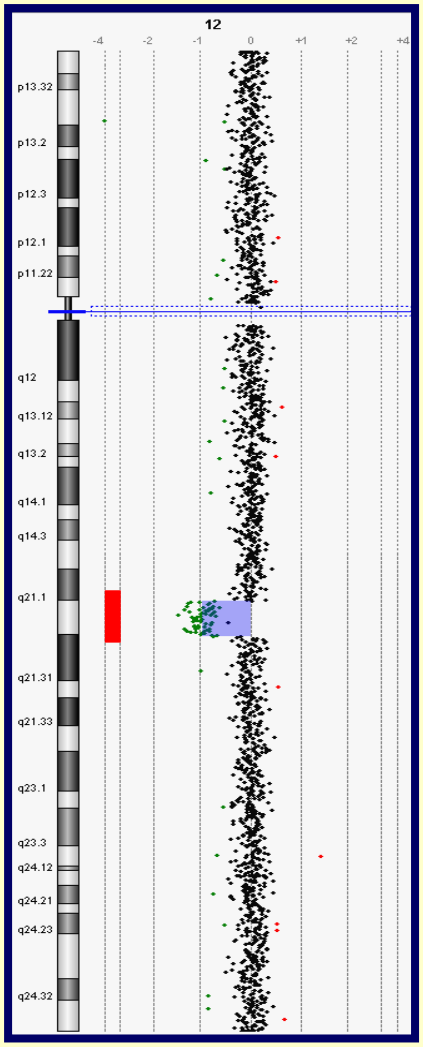


UBE3A

45 kb loss

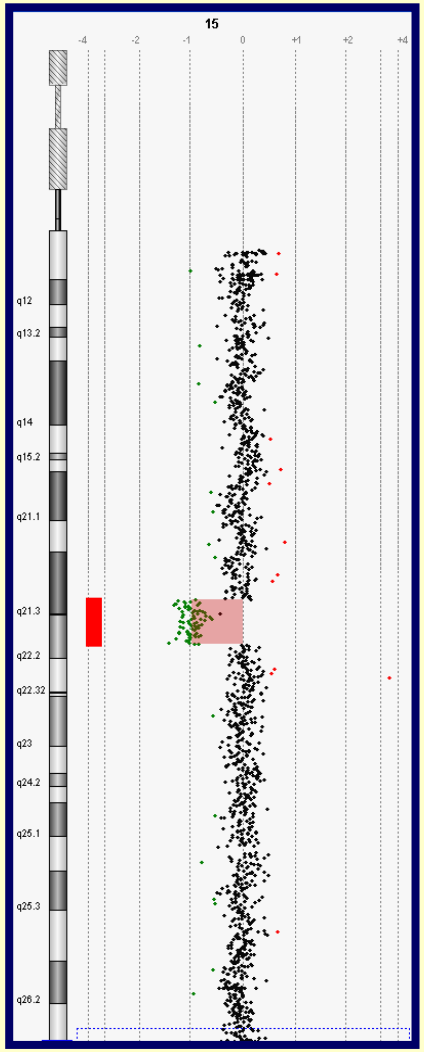
Whole Genome Coverage

Case 1



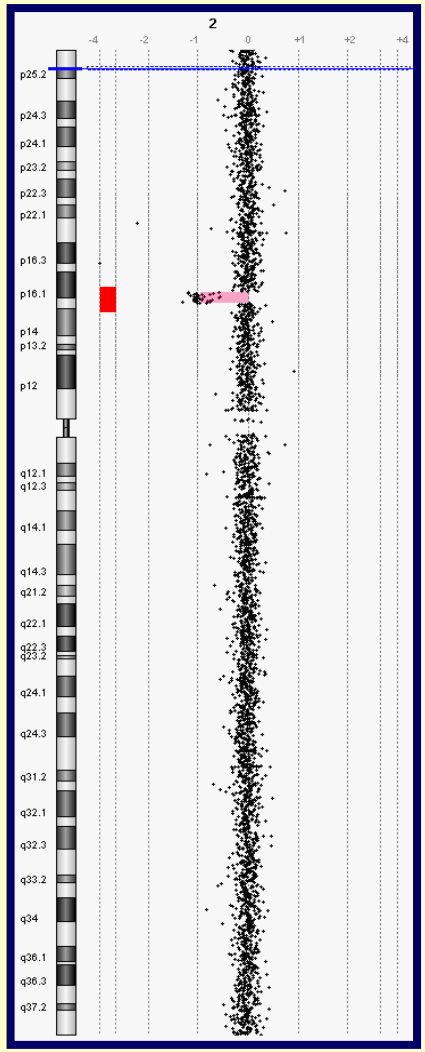
12q: 4.7 Mb deletion
~11 known genes

Case 2



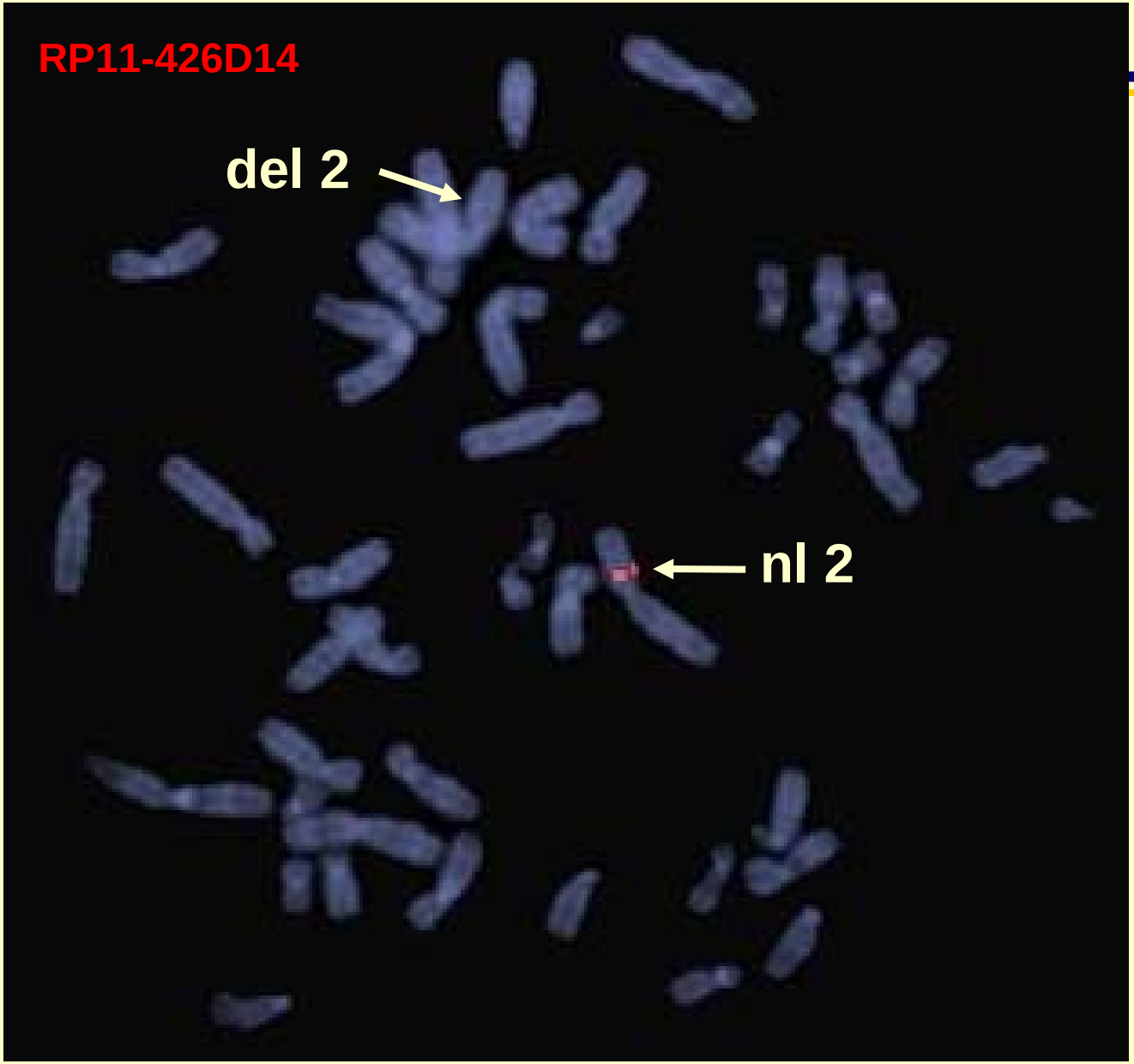
15q: 4.5 Mb deletion
~21 known genes

Case 3



2p: 3.0 Mb deletion
~ 12 known genes

FISH Confirmation – 2p deletion



32K BAC set

FISH = mech.

Targeted vs. Whole Genome Detection Rates

To date, more than 3,000 cases analyzed…

- **Abnormal detection rate: 18%**
- **Targeted coverage: 13%**
- **Whole genome coverage: 5%**

Whole genome coverage enhances the detection of clinically relevant cytogenetic imbalances

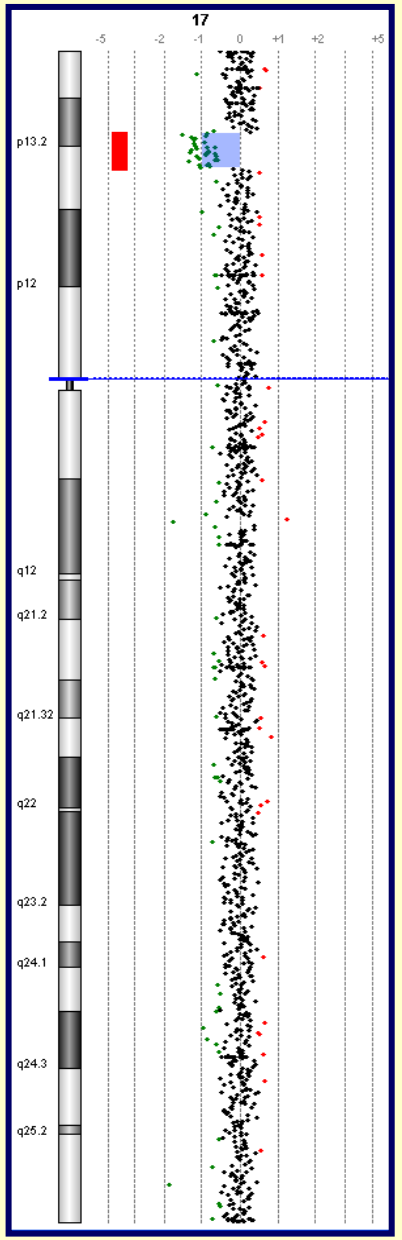
10% of patients who have karyotype first have a significantly delayed diagnosis!

Case 11

Referring Dx:

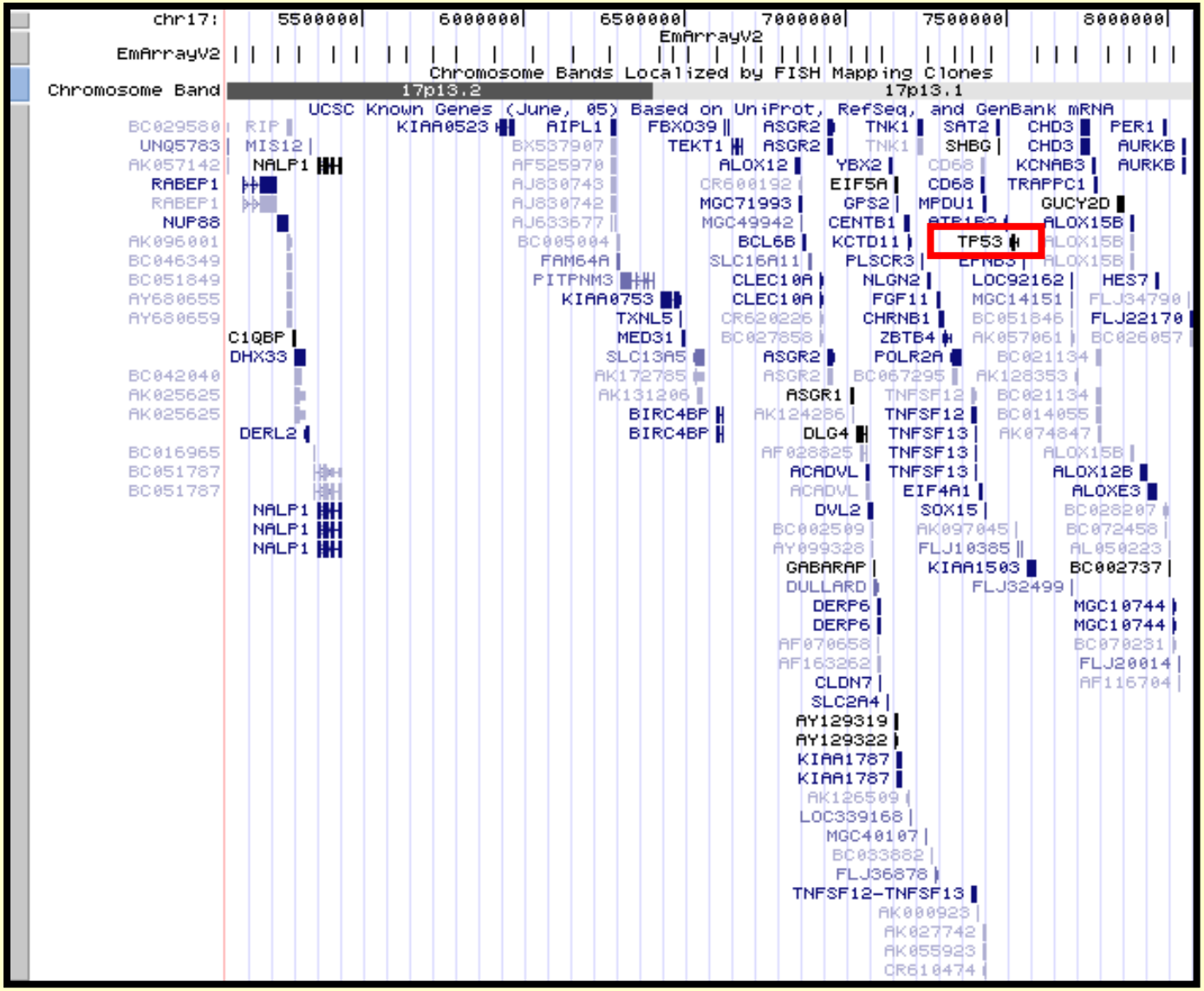
Dysmorphic features
Developmental delay
Hypotonia
Hypoplastic penis

17p: 2.3 Mb deletion



Case 11

Loss of 17p13.2p13.1: ~2.3Mb



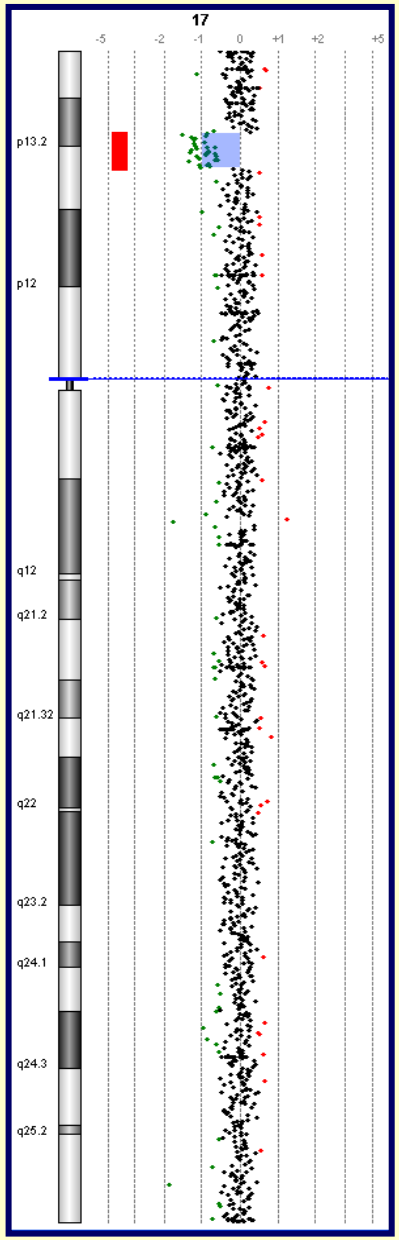
Cancer Susceptibility

Referring Dx:
Dysmorphic features
Developmental delay
Hypotonia
Hypoplastic penis

17p: 2.3 Mb deletion

p53 loss = Li-Fraumeni syndrome, high cancer risk

Adam et al., J Pediatrics, Jan., 2009
Other cases: *RB1*, *VHL*, Peutz-Jeghers

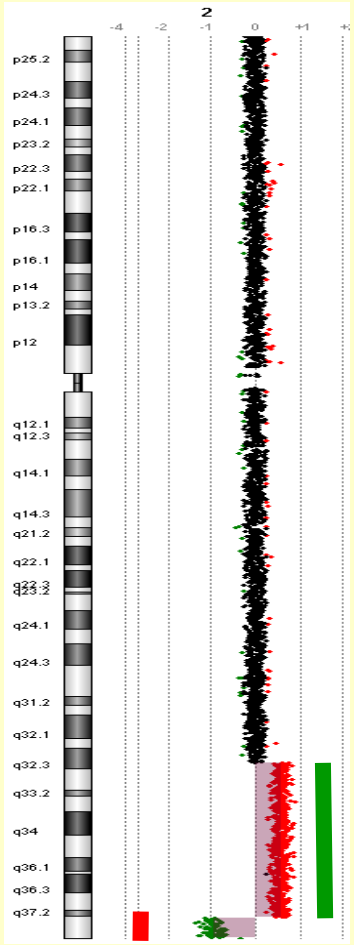


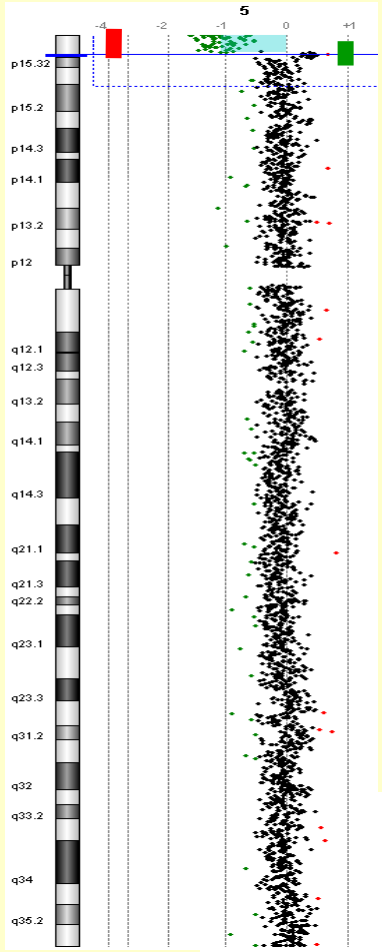
Mechanisms of Chromosome Rearrangements

Terminal telomere deletions with adjacent duplications – pre-meiotic breakage-fusion-bridge cycles after random breakage

16 cases:

2q x 2
4p
5p
6q
8p
9p x 3
10q
15q
18p
18q
22q x 3





Mechanisms of Chromosome Rearrangements

Breakpoint analysis: Random or specific mechanism?

Examined 54 cases with copy number imbalances (300 kb-10 Mb in size) with known inheritance:

15% mediated by flanking segmental duplications *(NAHR)*

85% were not associated with seg dups and most likely represent *random chromosome breakage*

C. Lee et al.: 7% of CNVs are associated with NAHR; majority are random

Current Status of Cytogenetic Array Testing

- **Multiple platforms**
  - **BAC vs. oligo**
  - **aCGH, SNP, beadchip**

  All detect single copy loss and gain accurately

- **Variable design and content**
  - **Targeted + whole-genome**
  - **increasing number of clinical loci including Mendelian genes**

- **~300 cyto labs in U.S.**
  - **? need/want 300 aCGH designs**

International aCGH Workshops (https://isca.genetics.emory.edu)

June 23-24, 2008 (Atlanta, GA)

30 attendees from U.S., Canada, UK and Brazil

Clinical Geneticists, Clinical Molecular & Cytogeneticists, Genomics & Bioinformatics

December 15-16, 2008 (Bethesda, MD)

60 attendees from U.S., Canada, UK, Belgium, Netherlands, Italy, Brazil

5 industry reps (Affymetrix, Agilent, BlueGnome, Nimblegen, OGT)

NCBI, NHGRI, NIMH, NICHD

Summary of 1st workshop (https://isca.genetics.emory.edu)

- Central, public database for clinical cyto array data (raw data files and normalized data) extremely valuable to clinical and research communities to rapidly identify pathogenic vs. benign CNCs
  - all de-identified data to achieve largest numbers, albeit with minimal clinical info
  - complete raw data and normalized data files
  - encourage informed consent and detailed clinical information for DECIPHER submission whenever possible

Summary of 1st workshop (https://isca.genetics.emory.edu)

- **Need more, high quality data on benign CNCs in normal controls, including mutation rate**

- ***Consensus that cytogenetic array should be 1st line diagnostic test for unexplained MR, MCA instead of karyotype (D. Miller, ms. in prep.)***

- **Need expert committee and evidence-based standards to make recommendations re:**
  - **clinical indications for testing**
  - **minimum standards for design, content, resolution, QA/QC**
  - **guidelines for interpretation and reporting**

2nd workshop (https://isca.genetics.emory.edu)

- New, higher quality data on normal controls from research community; culling of poor data from DGV

- NCBI received NIH IRB approval for de-identified data submission to dbGaP using “opt-out” mechanism of consent

- Increased international participation (Canada, UK, Netherlands, Belgium, Italy)

ISCA Steering Committee (https://isca.genetics.emory.edu)

Leslie Biesecker (NHGRI/NIH)
Nigel Carter (Sanger Institute, UK)
John Crolla (Salisbury, UK)
Evan Eichler (University of Washington)
Ada Hamosh (Johns Hopkins/OMIM)
David Ledbetter (Emory University)
Charles Lee (Harvard-Brigham & Women's)
Christa Martin (Emory University)
David Miller (Harvard-Boston Children's)
Nancy Spinner (CHOP)
Joris Vermeesch (Universiteit Leuven, Belgium)
Greg Peters (Australia)

International Public Database for Cytogenomic Array Data

- **Initially, minimal phenotypic data requirement but efforts to encourage detailed phenotypic data and submission to DECIPHER**

- **Will perform quality checks, summary tables, and public data release on quarterly basis**

  - **available to UCSC, Ensembl, DECIPHER, commercial vendors, local lab databases**

Proposal for a public database and evidence-based guidelines for design and interpretation

- **Technology platform and vendor neutral: BAC, oligo, beadchip**
  - **Common denominator is genome sequence coordinates for gains and losses**

- **Develop evidence-based guidelines for optimal design and interpretation**
  - **Minimum standards**

Current members of the Consortium: (agreed to public data sharing)

Alberta Children's Hospital
ARUP/University of Utah
Beth Israel Deaconess Medical Center
Children's Hospital of Philadelphia
Children's Memorial Hospital, Chicago
Cincinnati Children's Hospital
Credit Valley Hospital
Duke University
Emory University
GeneDx
Hamad Medical Corporation, Qatar
Henry Ford Hospital
Hospital for Sick Children, Toronto
Kaiser Regional Cytogenetics Lab
London Health Sciences Centre
Mayo Clinic
Mission Health, Fullerton Genetics Lab

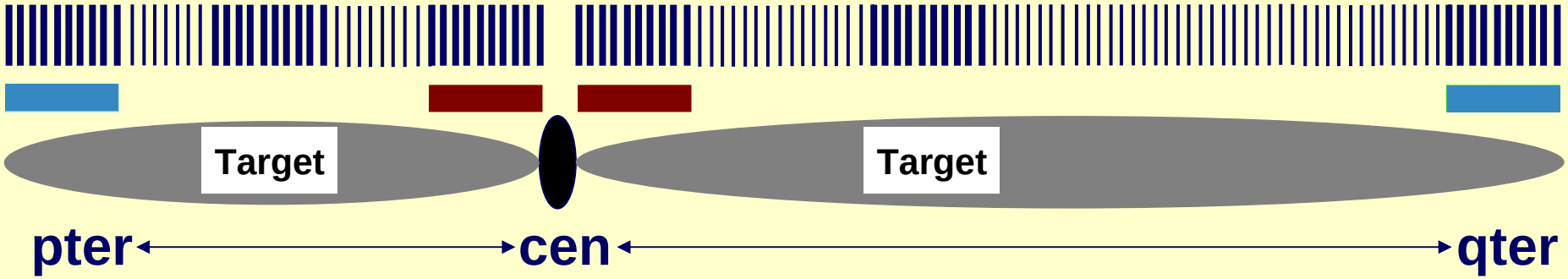
Current members of the Consortium: (agreed to public data sharing)

Montefiore Hospital
Northwestern Reproductie Genetics, Chicago
Stanford Hospitals and Clinics
Sudbury Regional Hospital
Texas Tech University
U. Mass. Memorial Medical Center
UMCG, Groningen, Netherlands
U. Alabama, Birmingham
U. Florida
U. Michigan
U. Nebraska
U. Oxford, UK
U. Rochester
U. Sao Paolo, Brazil
U. Wisconsin
U. Medical Center, Ljubljana
Wessex Regional Genetics Lab

ISCA "Community" array design (for labs that don't have own custom designs)

- Current array – 44k (4-plex), 105k (2-plex)
- ISCA drafts – 180k (4-plex)
  - 140k assigned; 40k available for customization
- Result of merging designs of existing arrays:
  Emory – Ledbetter/Martin
  GeneDx – Aradhya
  Salisbury, UK – Crolla/Barber
  Oxford, UK – Knight/Smith/Connell
  Dutch Consortium/Oxford design - Kok
  Belgium Consortium - Vermeesch
- …and continued improvements based on recommendations from ISCA Steering Committee

Whole-genome plus Targeted Community Array Design



Resolution

Telomere FISH clone

Unique centromere FISH clone

Clinically relevant targets (~500)

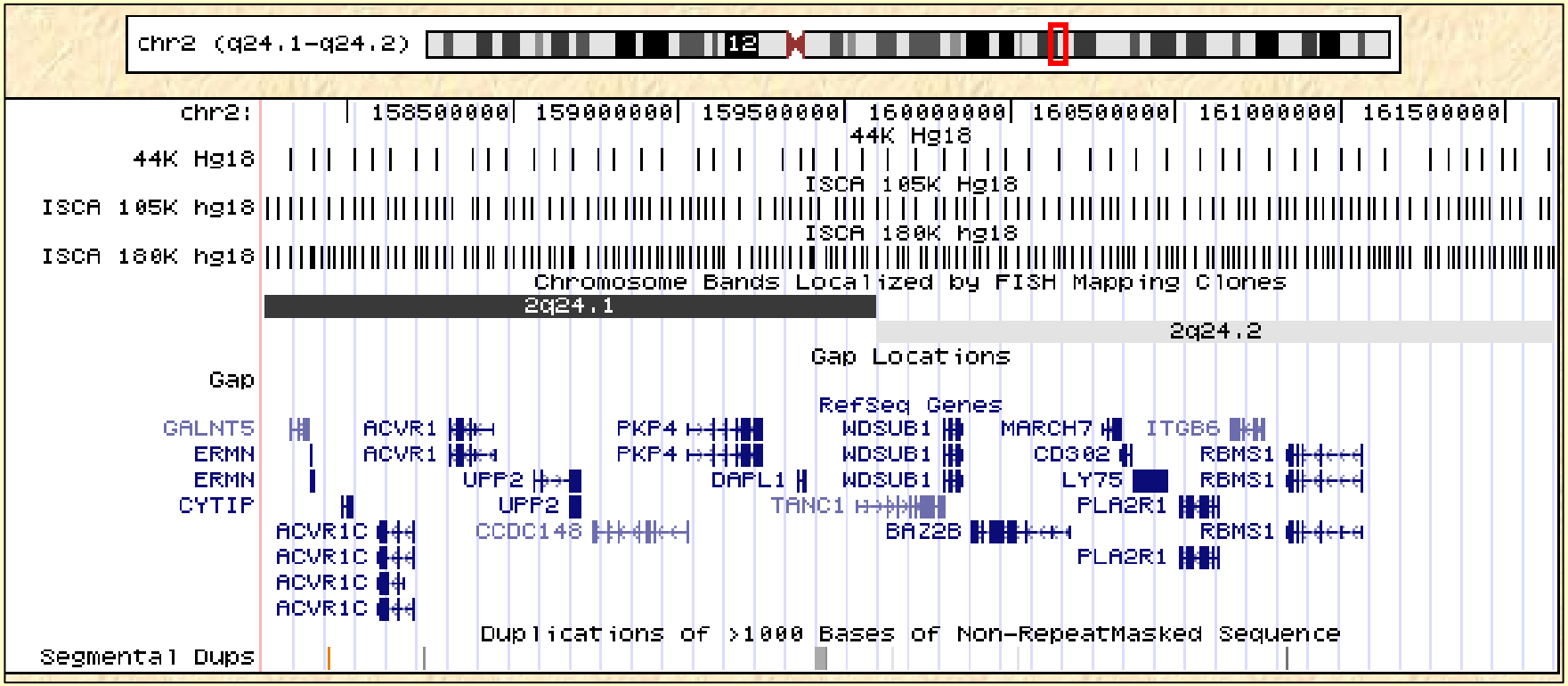
~30 - 50 kb

~25, 35 or 75 kb interval backbone (corresponds to 180K, 105K, 44K)

~100 - 250 kb

Backbone: 2q24

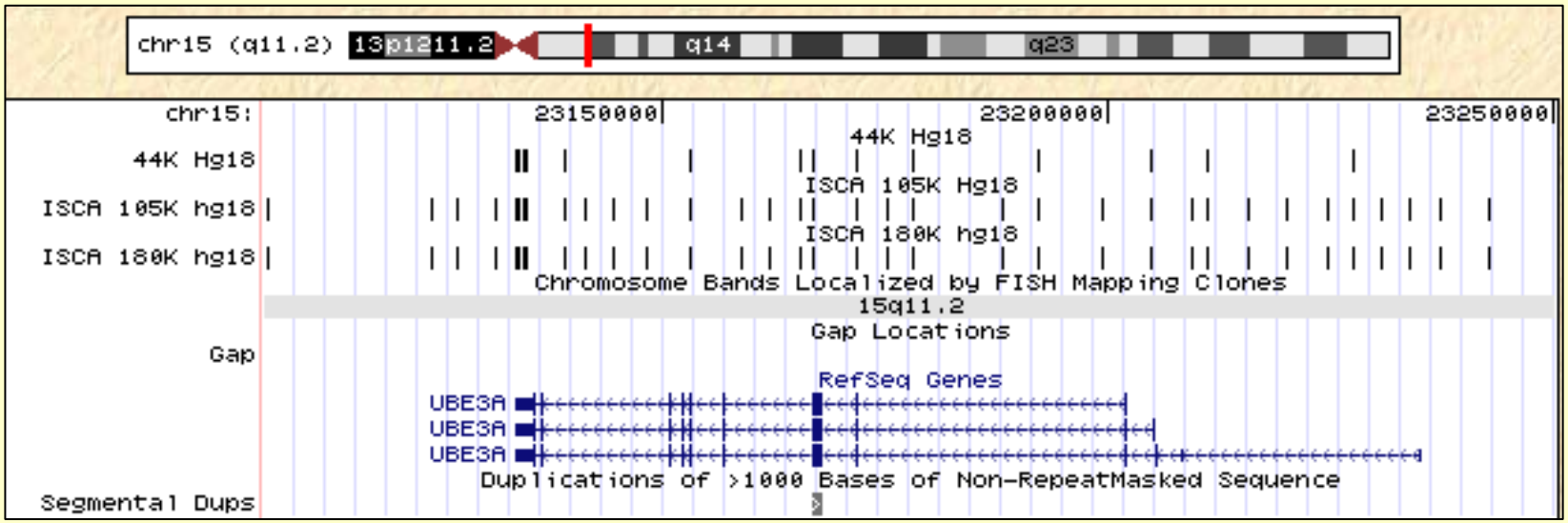
chr2:157,747,500-161,647,500 basepairs



- **44K: ~75 kb spacing (225 kb resolution)**
- **105K: ~35 kb spacing (105 kb resolution)**
- **180K: ~25 kb spacing (75 kb resolution)**

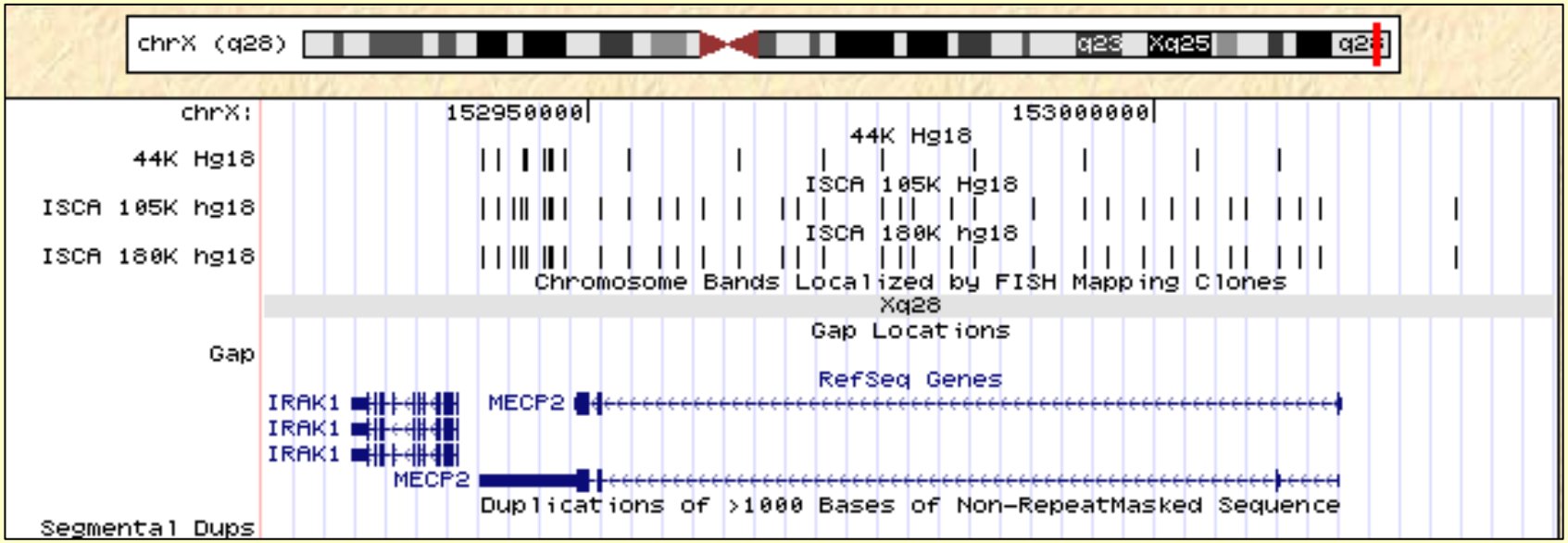
Targeted Gene: *UBE3A*

chr15:23,105,326-23,250,028 size: 145 kb



Targeted Gene: *MECP2*

chrX:152,921,476-153,035,363 size: 114 kb



Summary of Consortium Experience

- **Whole-genome oligo array clinical testing implemented April, 2007 (Emory and GeneDx)**

- **Over 25,000 clinical cases performed to date; Currently >500 cases/week.**
  - ***del 16p11.2 most common finding (1/300)***
  - ***1-2 new cases del 16p11.2 identified each week***

del 16p11.2 and autism

Human Molecular Genetics, 2008, Vol. 17, No. 4 **628–638**
doi:10.1093/hmg/ddm376
Advance Access published on December 21, 2007

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,*]

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 14, 2008 VOL. 358 NO. 7

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

The New York Times

THE DNA AGE

After DNA Diagnosis: 'Hello, 16p11.2. Are You Just Like Me?'

Samantha Napier, 14, left, and Taygen Lane, 4, share a rare genetic mutation.

By AMY HARMON
Published: December 28, 2007

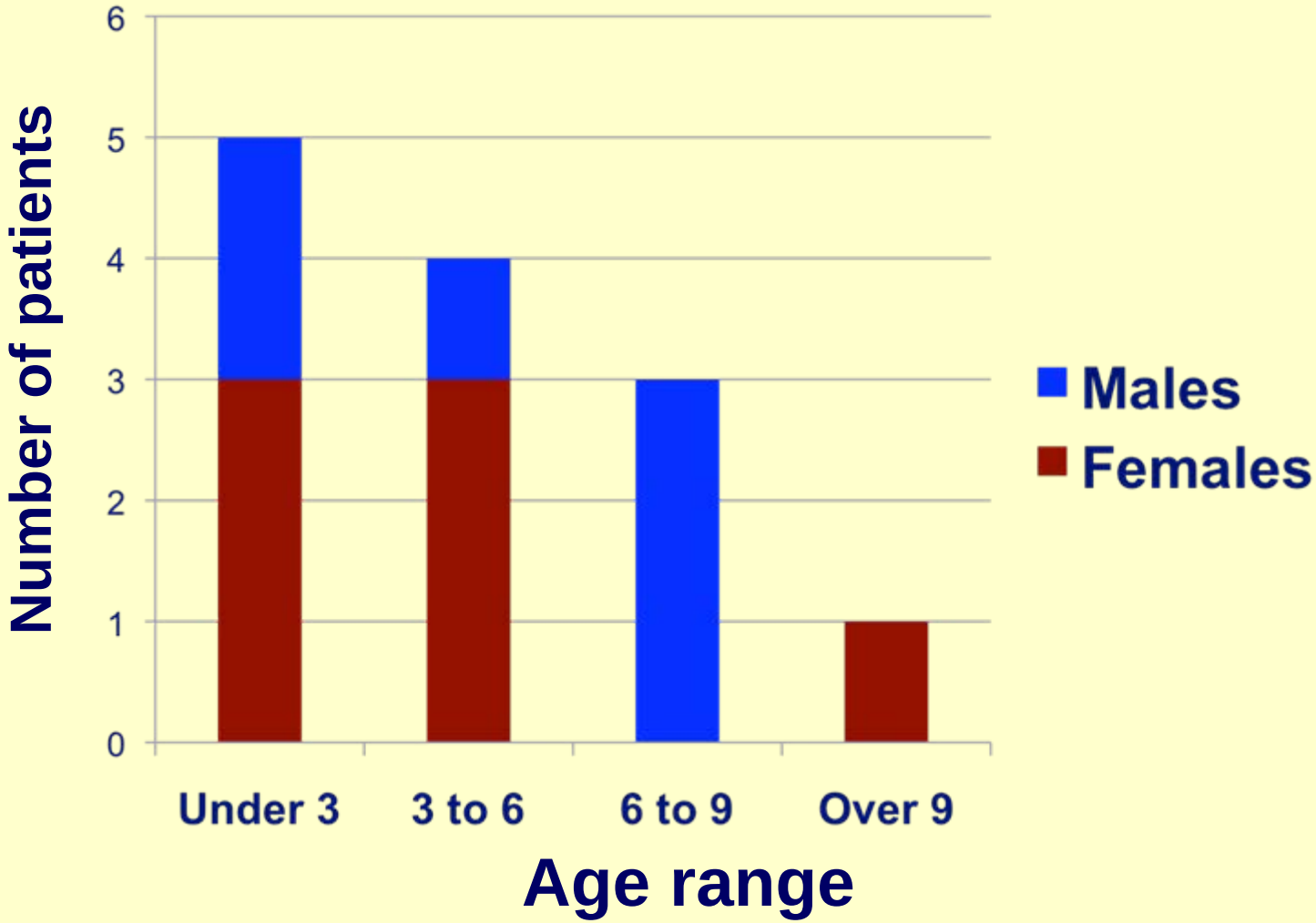
The girls had never met, but they looked like sisters.

The DNA Age

Articles in this series explore the impact of new genetic technology on American life.

There was no missing the similarities: the flat bridge of their noses, the thin lips, the fold near the corner of their eyes. And to the families of 14-year-old Samantha Napier and 4-year-old Taygen

del 16p11.2: clinical aCGH cases



Model for Genotype -> Phenotype Studies

- Patients
  - Identified on a clinical basis (“free”)
  - Early identification of at-risk children
  - Emory: 10 patients
  - GeneDx: 6 patients
  - 1/300 clinical aCGH tests = del 16p11.2
    - at least one new patient/week in consortium

Conclusions & Predictions

1) **Postnatal Cytogenetics- aCGH is much more sensitive that G-banded karyotype for postnatal, pediatric applications and *may soon become the primary genetic test* for children with unexplained developmental delay, mental retardation, birth defects, seizures, autism, etc.**

2) **Prenatal Diagnosis-**

NIH sponsored multicenter trial on prenatal aCGH underway to compare aCGH to G-banded karyotype. Results in ~2 years, but expect aCGH to win.

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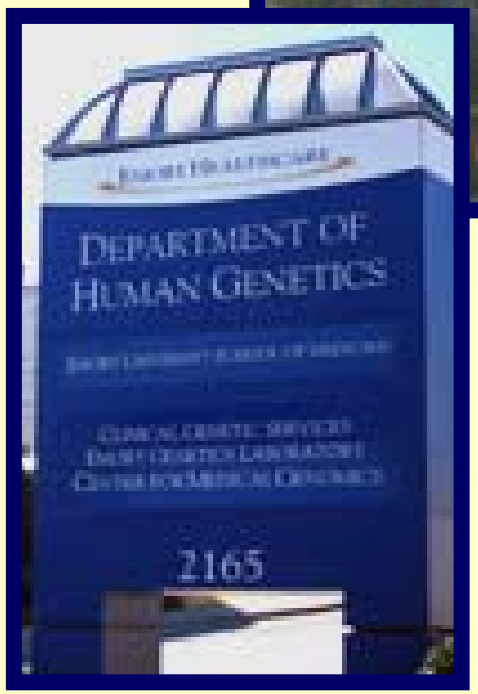

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