Clinical Exome Sequencing at Baylor Whole Genome Laboratory: Molecular Diagnosis and Disease Gene Discoveries

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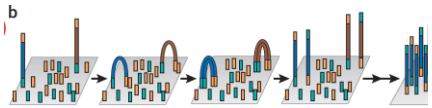
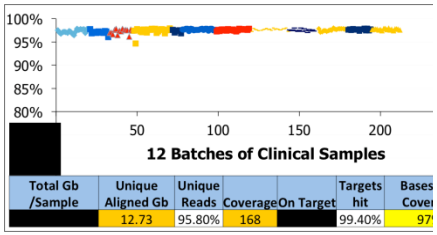
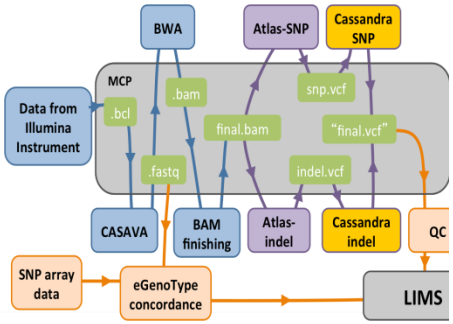
HGSC
HUMAN GENOME SEQUENCING CENTER







WGL
WHOLE GENOME LABORATORY

Baylor College of Medicine

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CMS
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MGL
MEDICAL GENETICS LABORATORIES

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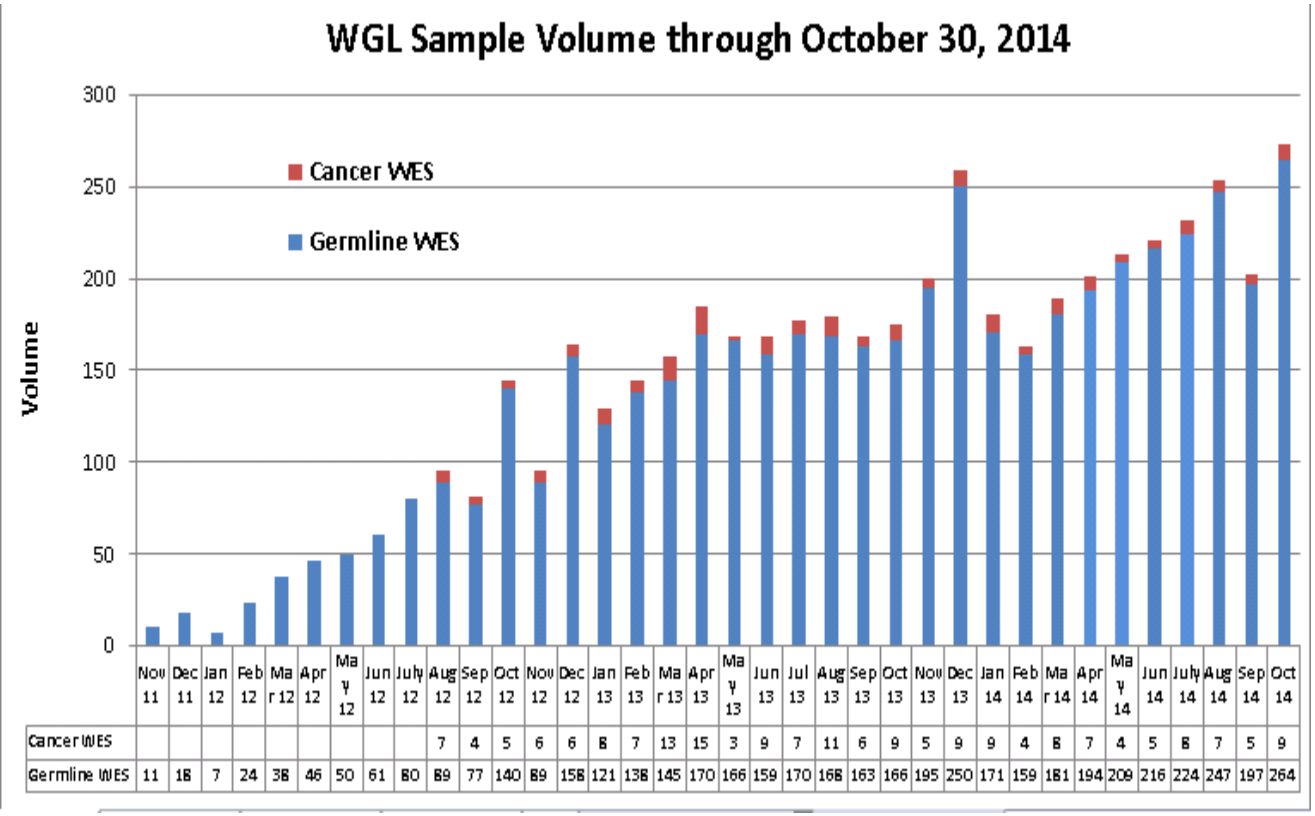
BCM WGL Launches Clinical Exome Sequencing Oct 2011



	Nov 11	Dec 11	Jan 12	Feb 12	Mar 12	Apr 12	May 12	Jun 12	July 12	Aug 12	Sep 12	Oct 12	Nov 12	Dec 12	Jan 13	Feb 13	Mar 13	Apr 13	May 13	Jun 13	Jul 13	Aug 13	Sep 13	Oct 13	Nov 13	Dec 13	Jan 14	Feb 14	Mar 14	Apr 14	May 14	Jun 14	July 14	Aug 14	Sep 14	Oct 14
Cancer WES										7	4	5	6	6	8	7	13	15	3	9	7	11	6	9	5	9	9	4	8	7	4	5	8	7	5	9
Germline WES	11	18	7	24	38	46	50	61	80	89	77	140	89	158	121	138	145	170	166	159	170	168	163	166	195	250	171	159	181	194	209	216	224	247	197	264

- ~ 5200 samples received, ~4200 cases finalized
- 85% peds; 15% adult
- Mostly neurologic
- In addition: skeletal disorders, pulmonary artery hypertension, cardiovascular dz
- Variety of referral sources – academic medical centers, private hospitals

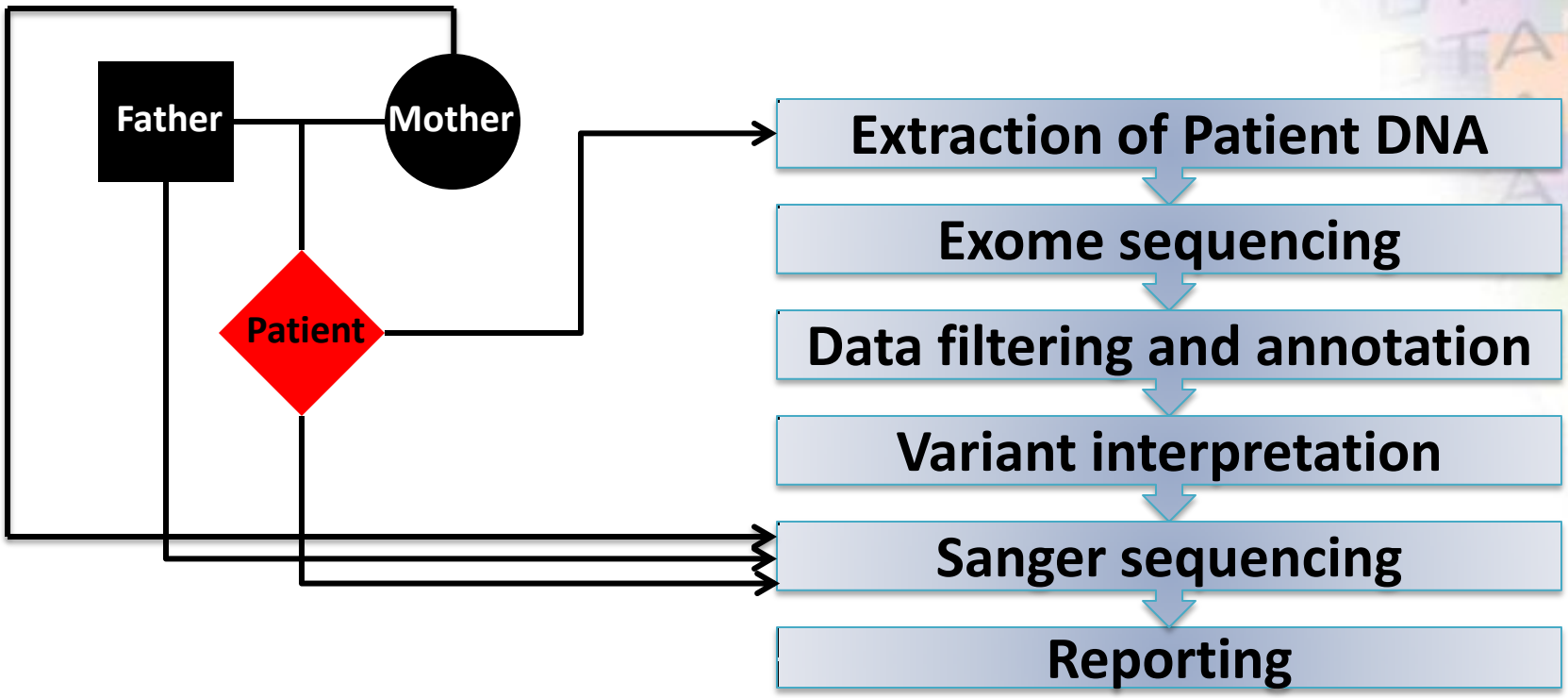
MONTHLY GERMLINE AND CANCER WES SAMPLES: ~230

N=4200 ~25% molecular Dx fits with clinical picture - Pts now given option to consent to research analysis for 75% no Mol. Dx. rendered

Clinical Exome Sequencing at WGL

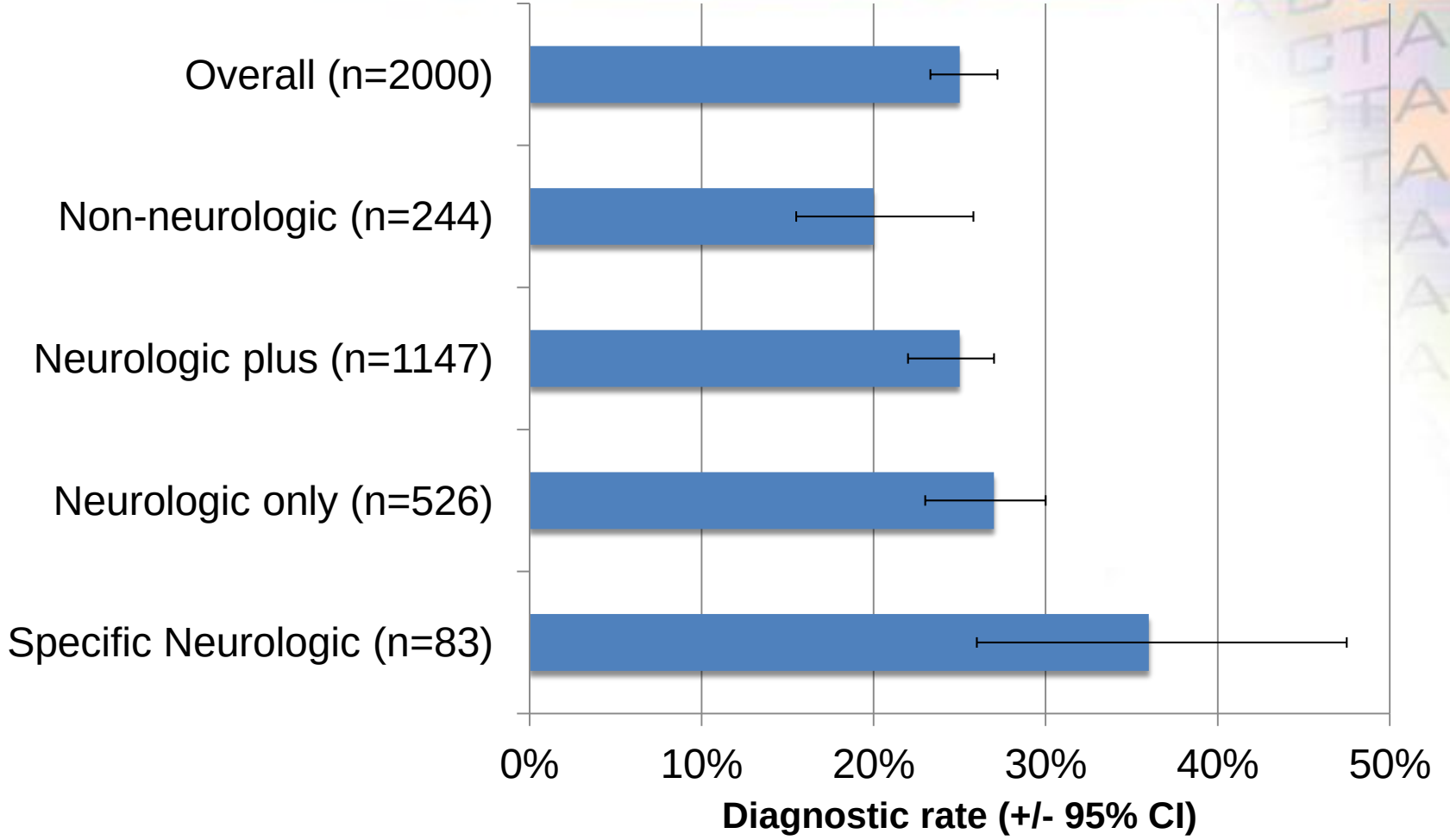
- Discoveries made
  - Diagnostic rates
  - Rare genetic events identified
  - New disease genes
- Lessons learned
  - Key elements of clinical exome

WES-Workflow for Proband Exome



Trio exome sequencing is also available from our laboratory now

Molecular Diagnosis Rate: Overall and by Phenotypic Groups

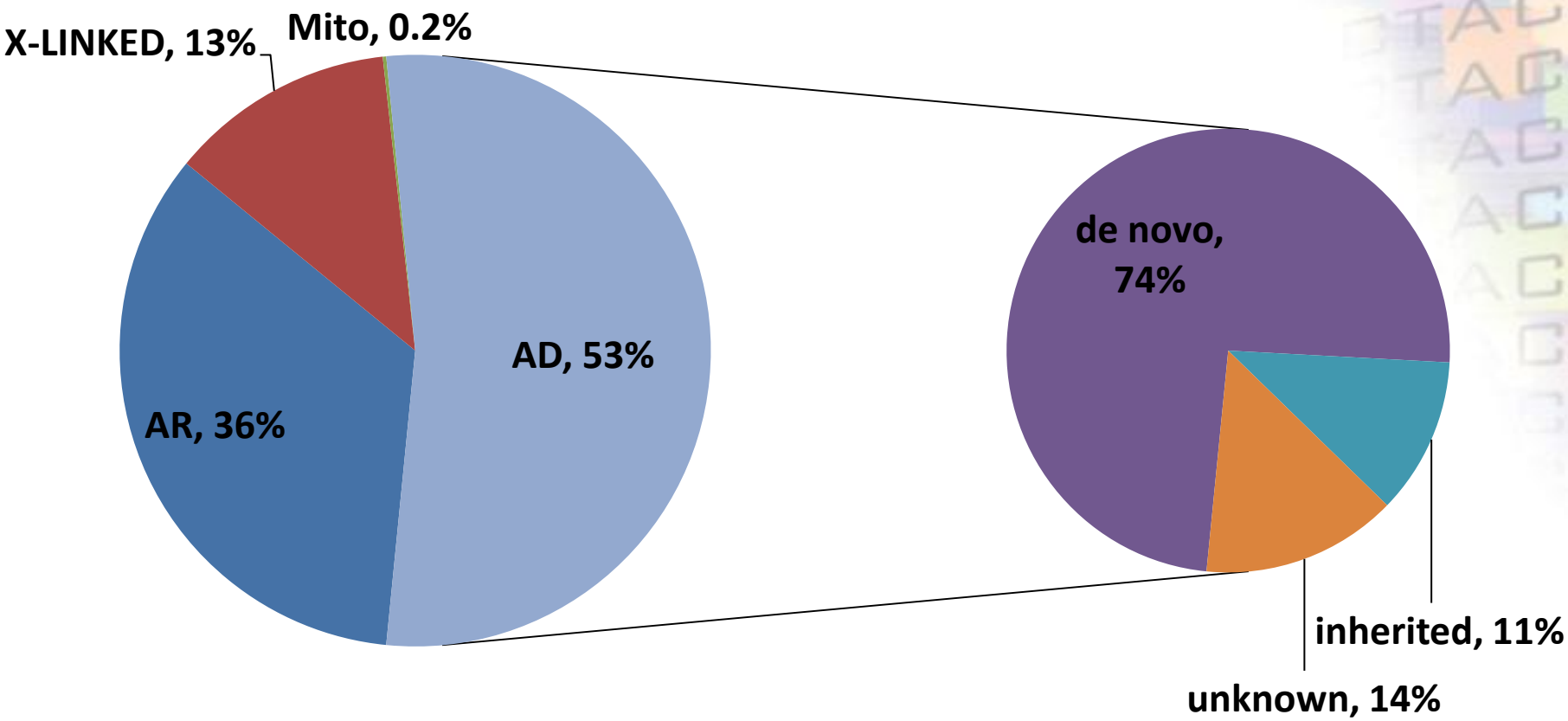


504/2000=25%

25% Diagnostic Rate Maintained for 3384 patients

Yang, et al., *JAMA,* 2014

Inheritance Manners among the 504 Positives in WES 2000



de Novo (AD: 74%; XL: 62%)

Findings against Textbook Expectations: Cases with Two Molecular Diagnoses (23/504) ~ 5%

Cases	AD	AD	AR	AR	X-linked	Two Diagnoses
1	*ANKRD11*	*ARID1B*				
2	*ASXL3*	*ENG*				
3	*CHD2*	*PRRT2*				
4	*CREBBP*	*PRICKLE2*				
5	*DYRK1A*	*KAT6B*				
6	*SCN1A*	*SMARCA2*				
7	*GLI2*	*IRF6*				AD+AD (7)
8	*DES*		*CLCN1*			
9	*KCNT1*		*TTN*			
10	*KIF5C*		*NRXN1*			
11	*KMT2A*		*TCIRG1*			
12	*NF1*		*GALNT3*			
13	*NF1*		*MEGF8*			
14	*SYNGAP1*		*MTFMT*			
15	*THRA*		*DGAT1*			AD+AR (8)
16	*ARID1B*				*GRIA3*	
17	*EFHC1*				*SMC1A*	
18	*FBN2*				*PQBP1*	
19	*TPM1*				*DMD*	AD+XL (4)
20			*AP4M1*	*ATM*		
21			*PAPSS2*	*TRDN*		
22			*RECQL4*	*XPC*		AR+AR (3)
23			*BBS10*		*PDHA1*	AR+XL (1)

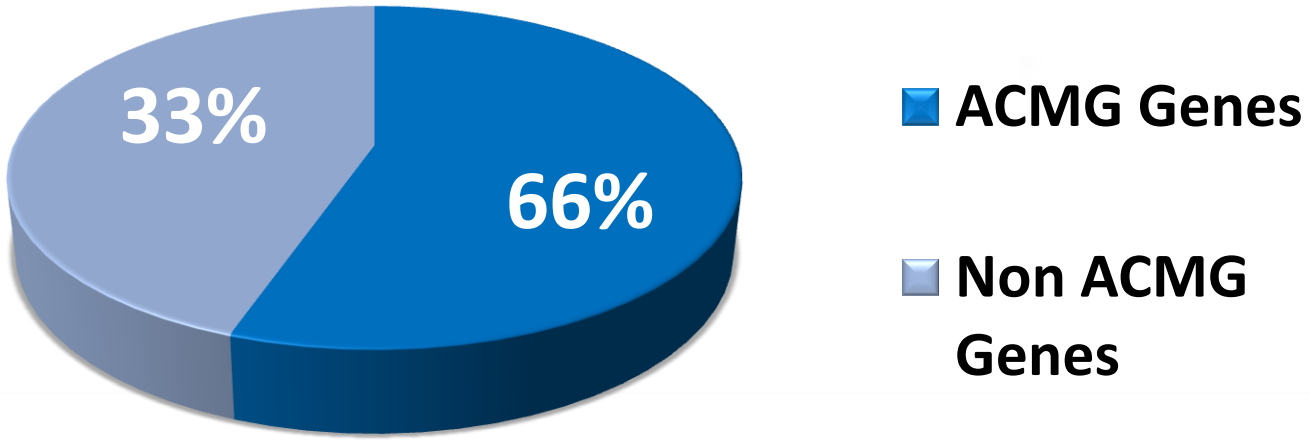
Findings against Textbook Expectations: Uniparental Disomy Detected in 5/504 Positive Cases

Case	Age/Sex	UPD	Isodisomy Type	Causal Genes/disease	Mat age/ Pat age
1	1.1/M	Mat UPD 2	Partial	*SCN9A* (epilepsy, insen. Pain)	36/41
2	9.6/M	Pat UPD 2	Complete	*CHRNG* (pterygium, lethal)	19/18
3	20/F	Pat UPD 9	Complete	*SIGMAR1* (ALS 16, juvenile)	32/28
4	4/M	Mat UPD 22	Complete	*PLA2G6* (neuraxonal dystrophy)	27/33
5	15/F	UPD 3 [a]	Complete	*SLC25A38* (anemia, sideroblastic)	n.a./n.a. [a]

[a] Parental samples not available

Medically actionable incidental findings (95/2000: ~5%)

- Unrelated to the phenotype but with immediate implications
- ACMG recommended genes (56): Cancer predisposition, Cardiomyopathy, Long QT
- Non-ACMG: G-6-PD, Fabry disease, mt mutation conferring risk for hearing loss



Examples of New Gene Discoveries Leading to Updated Reporting

Case	Date-Original Report	Date-Disease Gene Discovery	Date-Updated Report	Gene	Disease
1-3	Dec 2012	Sep 2013	Oct 2013	*MAGEL2*	Prader-Willi-like, intellectual disability, autism
4	Feb 2013	Sep 2013	Sep 2013	*FBXL4*	Mitochondrial Encephalopathy
5-6	Oct 2012	Dec 2012	Jul 2013	*WDR45*	Neurodegeneration with brain iron accumulation 5
7	Mar 2013	May 2013	Jul 2013	*DEPDC5*	Familial focal epilepsy with variable foci
8	April 2012	Jun 2012	Jul 2013	*SERAC1*	3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome
9	Dec 2013	May 2014	May 2014	*ADHC1*	Xia-Gibbs syndrome
10	Jan 2013	Oct 2014	Oct 2014	*PURA*	Neonatal hypotonia, seizures and encephalopathy (5q31.3 microdeletion syndrome)

Clinical Exome Sequencing on Proband

Oct 2011-Jun 2012
First 250 Samples
N Engl J Med.
Oct. 2013

Jun 2012-Nov 2013
Additional 2000 Samples
JAMA 2014
Oct. 2014

Currently
~200 samples/month
WES Version 3

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

Yaping Yang, Ph.D., Donna M. Muzny, M.Sc., Jeffrey G. Reid, Ph.D., Matthew N. Bainbridge, Ph.D., Alecia Willis, Ph.D., Patricia A. Ward, M.S., Alicia Braxton, M.S., Joke Beuten, Ph.D., Fan Xia, Ph.D., Zhiyv Niu, Ph.D., Matthew Hardison, Ph.D., Richard Person, Ph.D., Mir Reza Bekheirnia, M.D., Magalie S. Leduc, Ph.D., Amelia Kirby, M.D., Peter Pham, M.Sc., Jennifer Scull, Ph.D., Min Wang, Ph.D., Yan Ding, M.D., Sharon E. Plon, M.D., Ph.D., James R. Lupski, M.D., Ph.D., Arthur L. Beaudet, M.D., Richard A. Gibbs, Ph.D., and Christine M. Eng, M.D.

Research

Original Investigation

Molecular Findings Among Patients Referred for Clinical Whole-Exome Sequencing

Yaping Yang, PhD; Donna M. Muzny, MS; Fan Xia, PhD; Zhiyv Niu, PhD; Richard Person, PhD; Yan Ding, MD; Patricia Ward, MS; Alicia Braxton, MS; Min Wang, PhD; Christian Buhay, BS; Narayanan Veeraraghavan, PhD; Alicia Hawes, BS; Theodore Chiang, MS; Magalie Leduc, PhD; Joke Beuten, PhD; Jing Zhang, PhD; Weimin He, PhD; Jennifer Scull, PhD; Alecia Willis, PhD; Megan Landsverk, PhD; William J. Craigen, MD, PhD; Mir Reza Bekheirnia, MD; Asbjorg Stray-Pedersen, MD, PhD; Pengfei Liu, PhD; Shu Wen, PhD; Wendy Alcaraz, PhD; Hong Cui, PhD; Magdalena Walkiewicz, PhD; Jeffrey Reid, PhD; Matthew Bainbridge, PhD; Ankita Patel, PhD; Eric Boerwinkle, PhD; Arthur L. Beaudet, MD; James R. Lupski, MD, PhD; Sharon E. Plon, MD, PhD; Richard A. Gibbs, PhD; Christine M. Eng, MD

Clinical Exome Sequencing at WGL

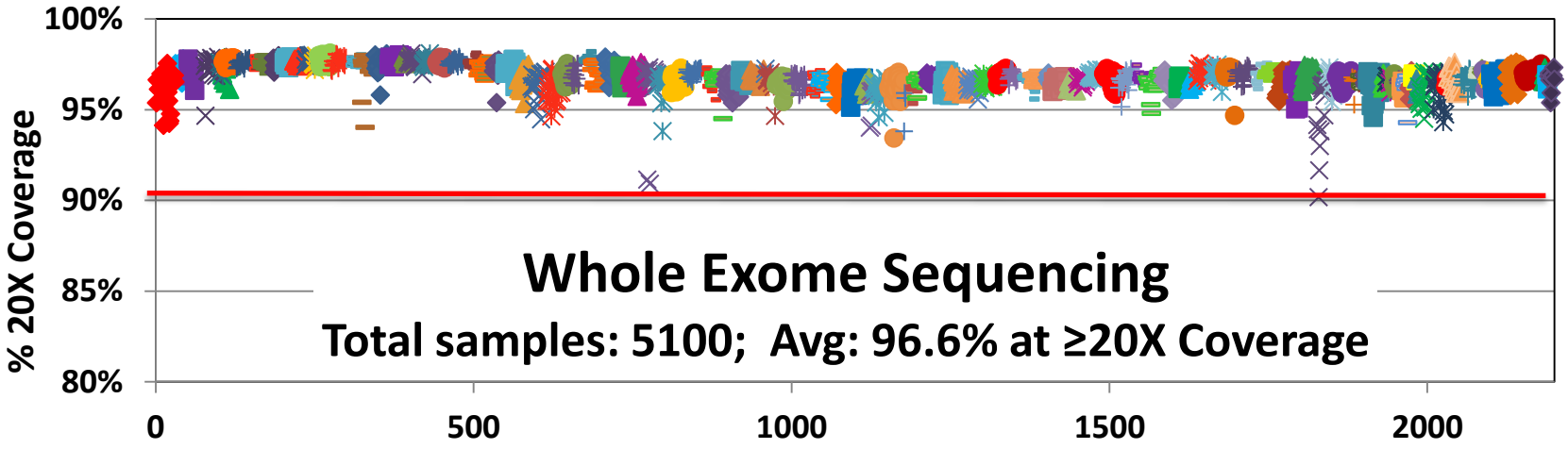
- Discoveries made
  - Diagnostic rates
  - Rare genetic events identified
  - New disease genes
- **Lessons learned**
  - **Key elements of clinical exome**

Key Elements of Clinical Exome

- **Optimization wet lab assays**
  - **Improve exome coverage and turn-around time (TAT)**
- Variant interpretations and classifications
  - SNVs, CNVs and AOH analyses
  - Don’t stop at one diagnosis, the patient could have blended phenotypes resulting from two single gene defects
  - Incorporating clinical expertise in exome reporting
- Building and sharing knowledge database
- New disease gene discoveries

Wes Version 1: 'WGL' – VCRome2.1 is 'just right'

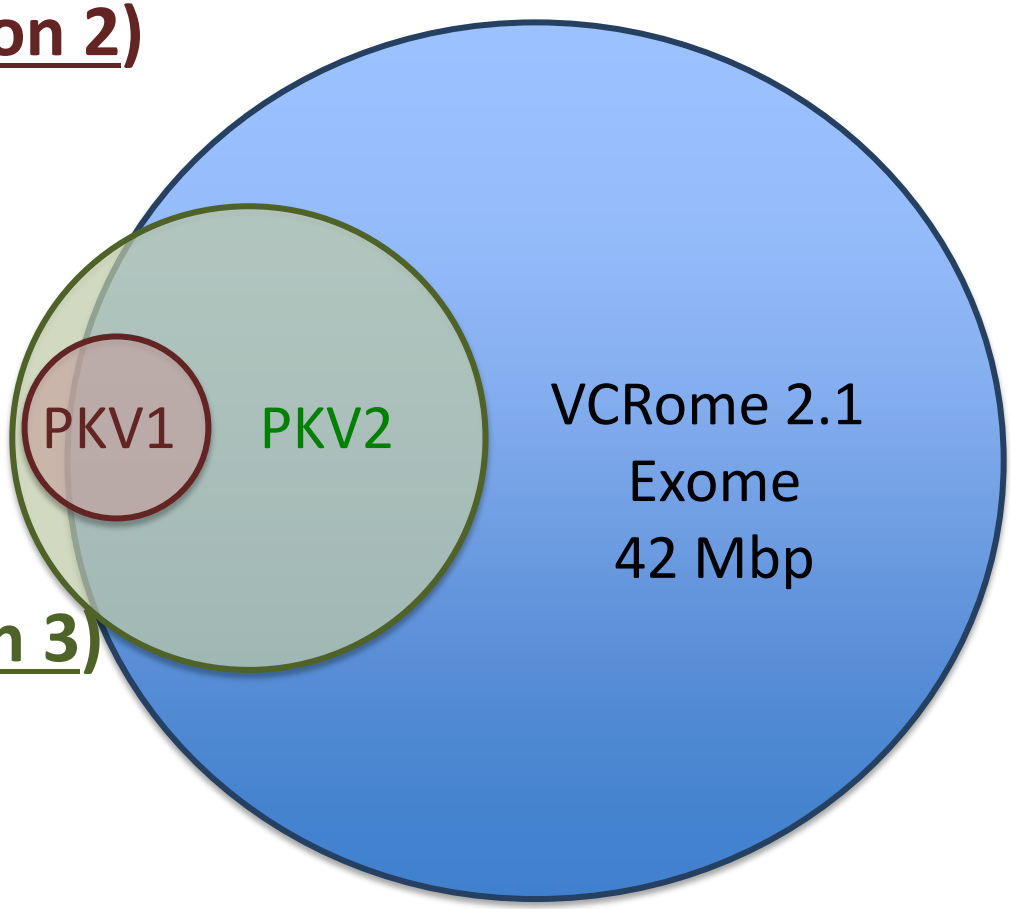
- **Coding Exons from:** Vega, CCDS, RefSeq,
- **Predicted coding exons from:** Contrast and GenScan.
  - 197K targets, 42Mb genomic region; NimbleGen Rebalanced x2



Observation:
Some regions not covered….still need 'polishing'!
What about comparison with clinical panels?
What about 'Medical Exome'

Exome “Spike-in” design content

Spike-in PKv1 (WES Version 2)
1977 Genes (0.220 Mbp)
GeneTests
21 Clinical Panels

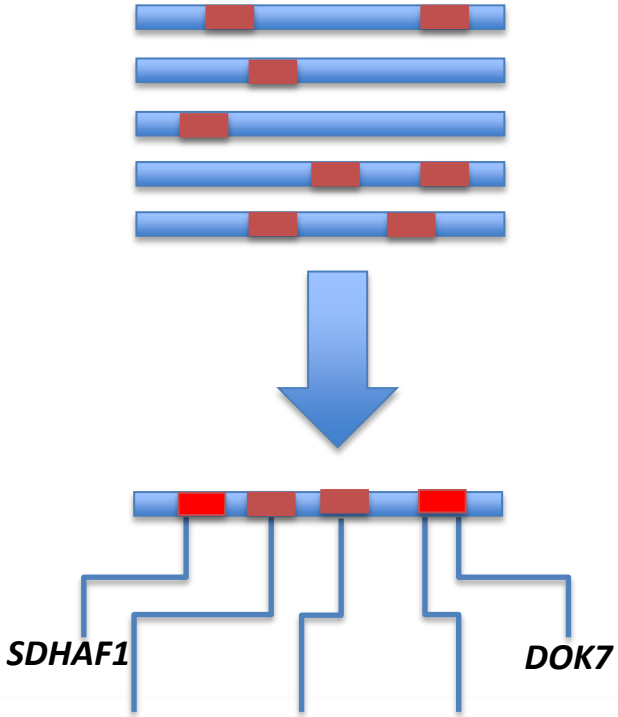


Spike-in PKv2 (WES Verson 3)
3643 Genes (2.5 Mbp)
PKv1 design
OMIM
Selected Cancer Genes
Solved Clinical Cases

By Donna Muzny et al.

Evaluation of ~100 Positive Samples Tested by WES Version 2

- Would the molecular diagnoses for the 100+ cases have been made definitively if the samples had been tested by WES Version 1?

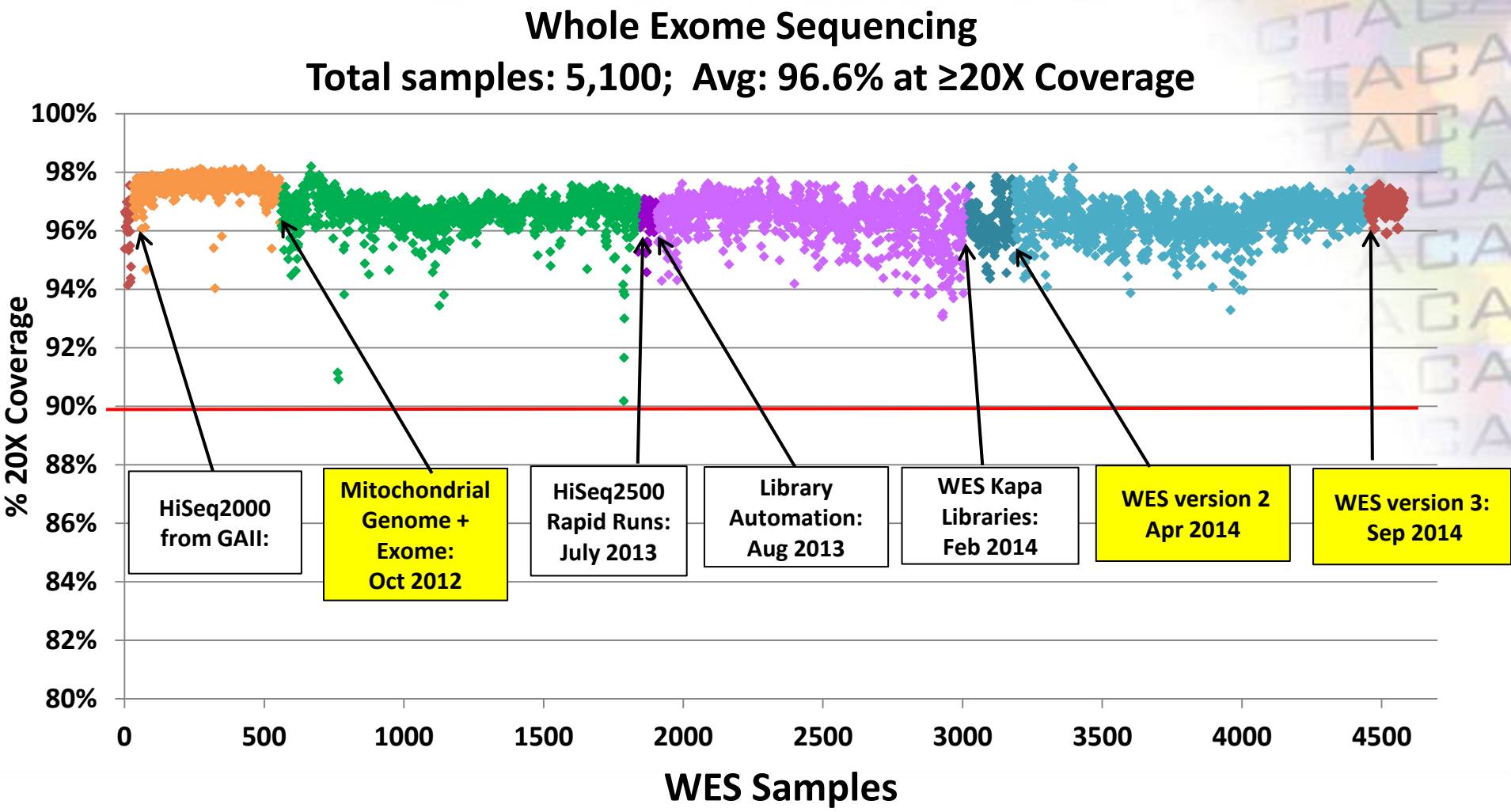


① Start with causal variants in the 100+ cases tested by **WES V2**
② Identify genomic coordinates for the causal variants
③ Plot sequence coverage across target regions of **WES V1**
④ Flag regions where coverage <20X

WES Version 1 Would Have Missed Molecular Diagnoses for Three Cases

- 1.7 yr old male
  - ***SDHAF1*** Mitochondrial complex II deficiency [MIM:252011]
  - Homozygous pathogenic variant: **c.156C>A (p.Y52X), 1/1:50:1:51**
- 19.3yr old male
  - ***DOK7*** Familial limb-girdle myasthenia (LGM) [MIM: 254300]
    Fetal akinesia deformation sequence [MIM:208150]
  - Compound heterozygous **c.1138dup (p.A380fs), 1/0:45:40:85,** and c.1476_1485dup (p.G496fs),
- 13 year old female
  - ***ADCY5,*** Dyskinesia, familial, with facial myokymia [MIM 606703]
  - **c.1253G>A (p.418Q), 0/1:8:18:26**, de novo

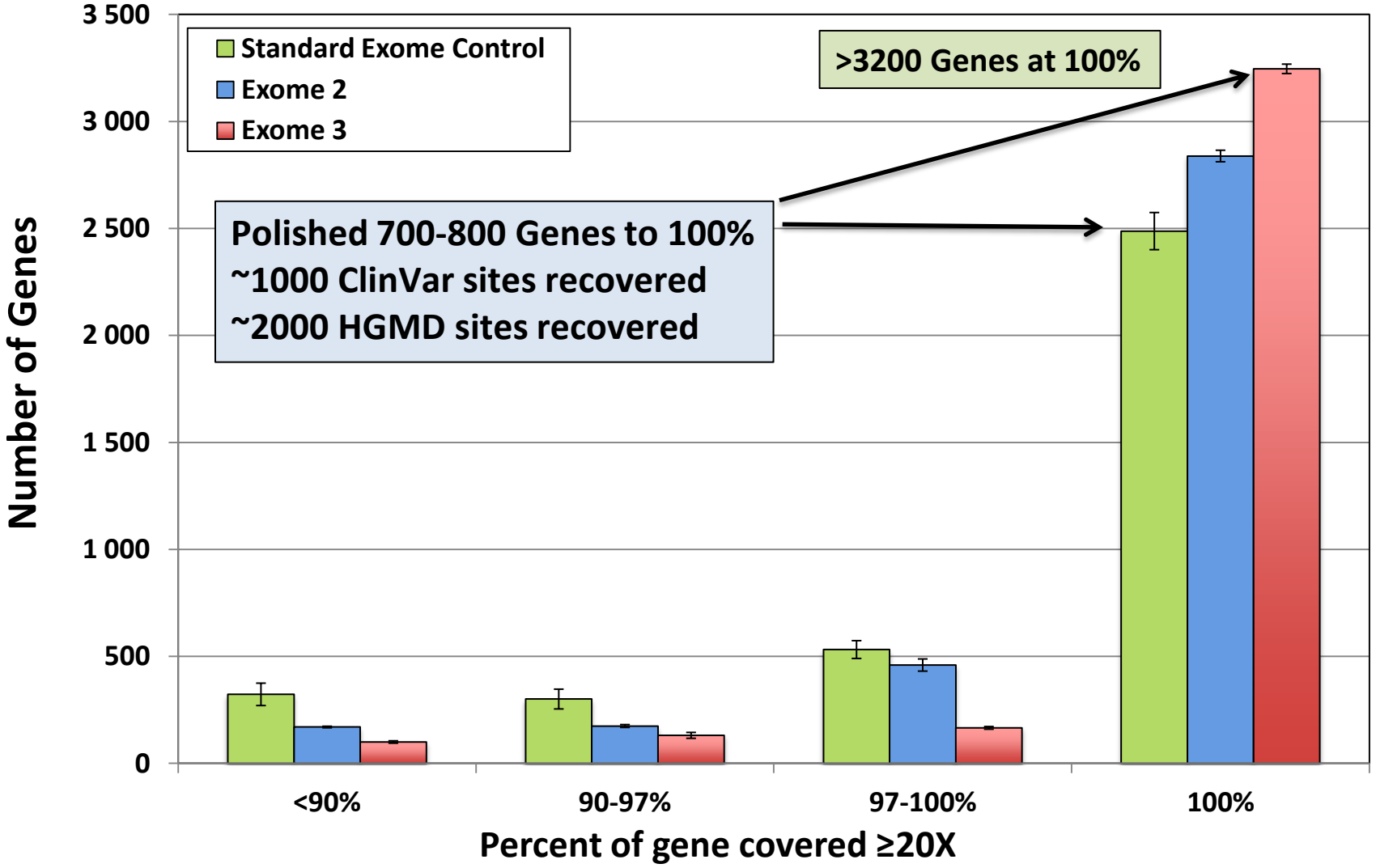
WGL Whole Exome Sequencing Historical Summary



PKv2 (WES Version 3) Design Performance

Includes GeneTests and OMIM (n=3643)

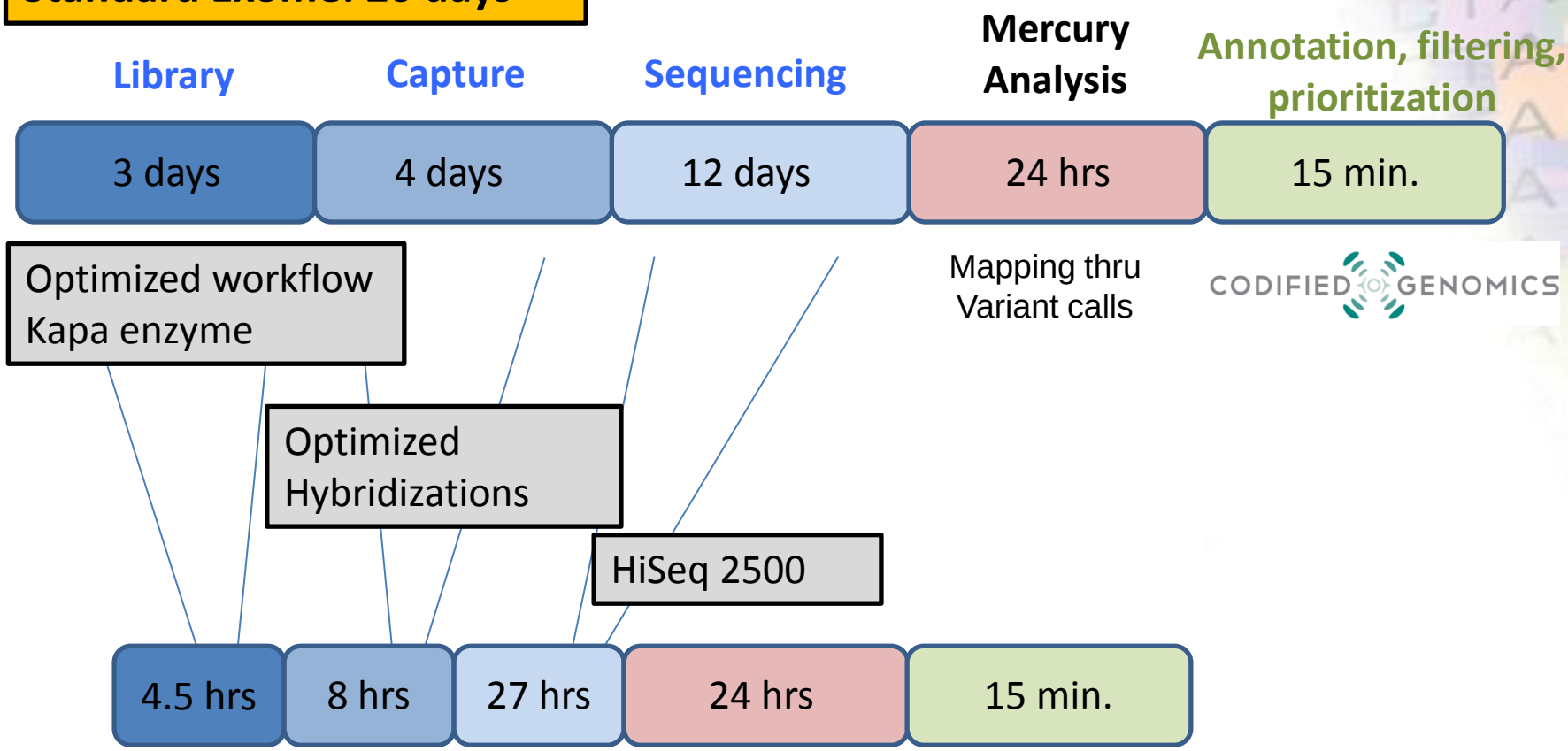
11Gbp, VCRome 2.1 exome + PKv2 Spike-in Design



By Donna Muzny et al.

Lightning Capture:
Reduced turnaround time in the wet lab

Standard Exome: 20 days



Demonstrated using three sample dataset, 11Gbp
>98% target bases at 20x;
>94% target bases at 40x;

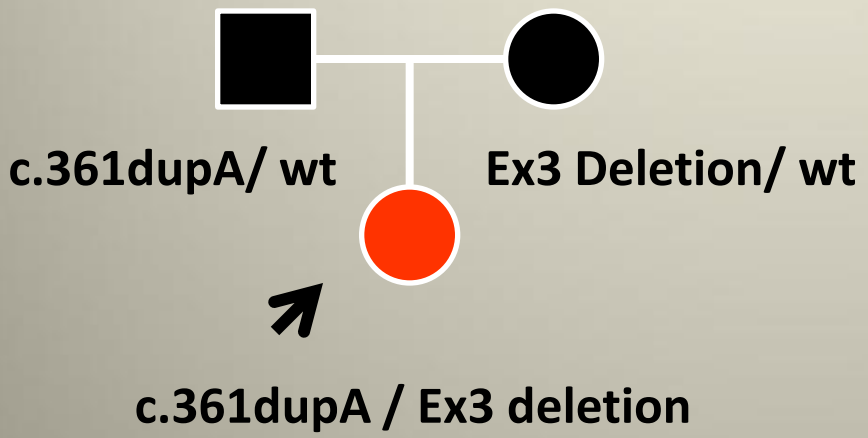
Lightning Exome: 64 hrs (<3 days)

By Donna Muzny et al.

Key Elements of Clinical Exome

- Optimization wet lab assays
  - Improve exome coverage and turn-around time (TAT)
- **Variant interpretations and classifications**
  - **Analyses of single nucleotide variants (SNVs), as well as copy number variants (CNVs) and absence of heterozygosity (AOH) regions**
  - Thorough data analyses
    - Explore all possible inheritance manners
    - Don’t stop at one diagnosis, the patient could have blended phenotypes resulting from two single gene defects
  - Incorporating clinical expertise in exome reporting
- Building and sharing knowledge database
- New disease gene discoveries

Rare Genetic Events: SCID due to compound heterozygous *IL7R* Mutations (SNV+CNV) Detected by WES and CMA



Sporadic case of SCID: CNV + SNV = Aut Recess SCID

The case is solved combining genomic analysis by BOTH WES and CMA

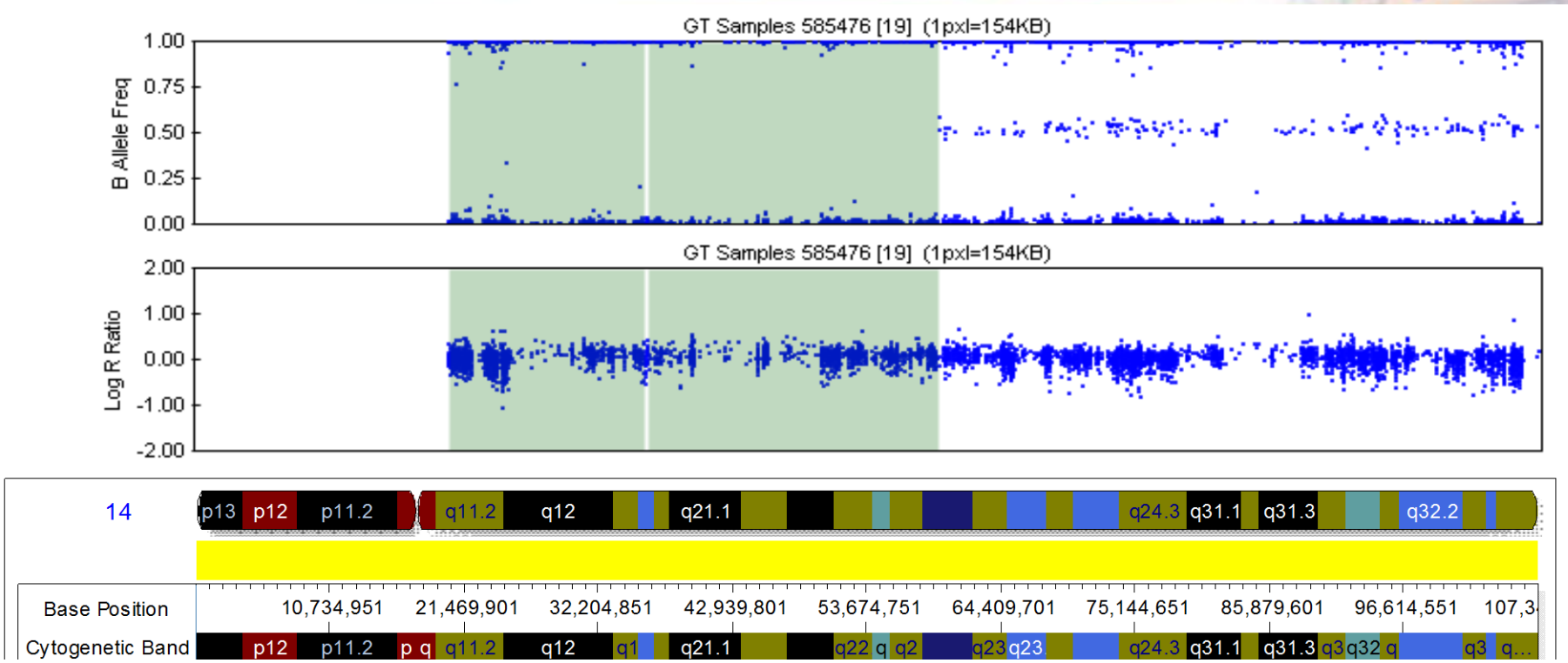
Case example

- 5 year old male
- Father is 39 yrs old, mother is 27 yrs old

- Clinical Presentation:
  - Global developmental delay (a history of hypotonia, rolled at 12 mo, sat at 12 mo, walked at 2 yr, first words at 2.5 yr, still receives ST)
  - Overweight (at 4y 8 mo, weight 90-95th ile)
  - Mild joint laxity
  - Genital anomalies
  - Mild facial dysmorphisms
  - Behavioral problems (aggression)

- Tested negative for Prader-Willi Syndrome (PWS)

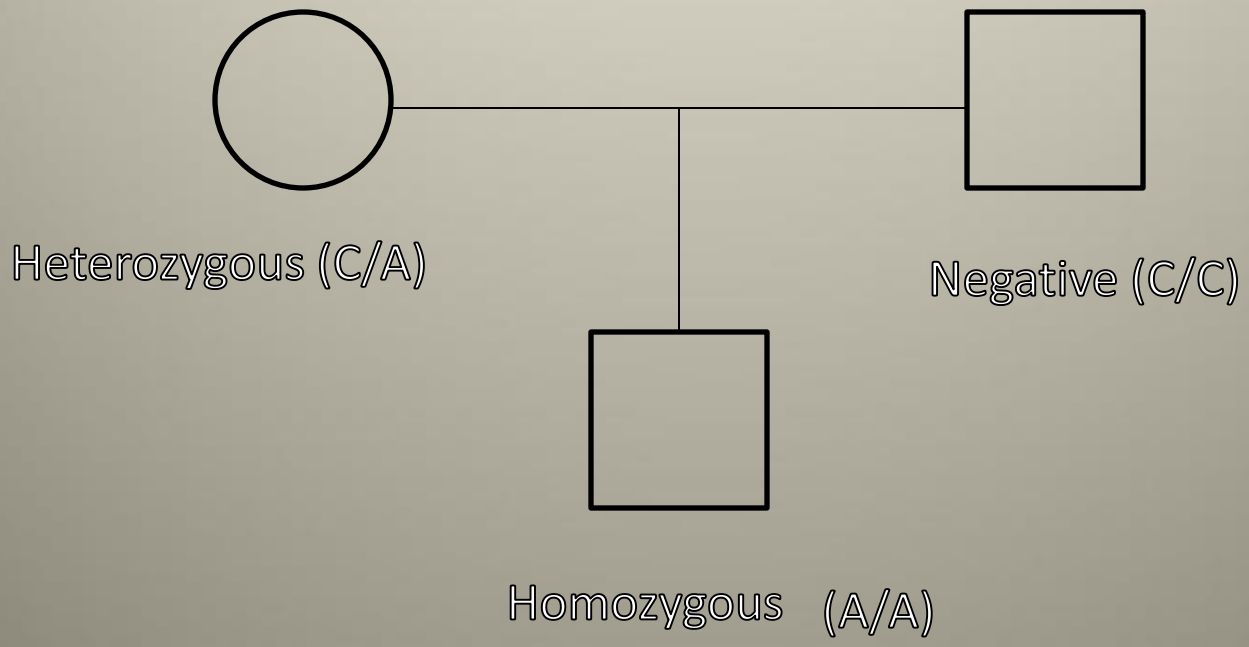
Absence of Heterozygosity (AOH) on chromosome 14



Concurrent Illumina HumanExome-12v1 (cSNP) array analysis revealed contiguous regions of copy neutral Absence of Heterozygosity (AOH) on chromosome 14 (approximate 39 Mb, 14q11.2-14q22)

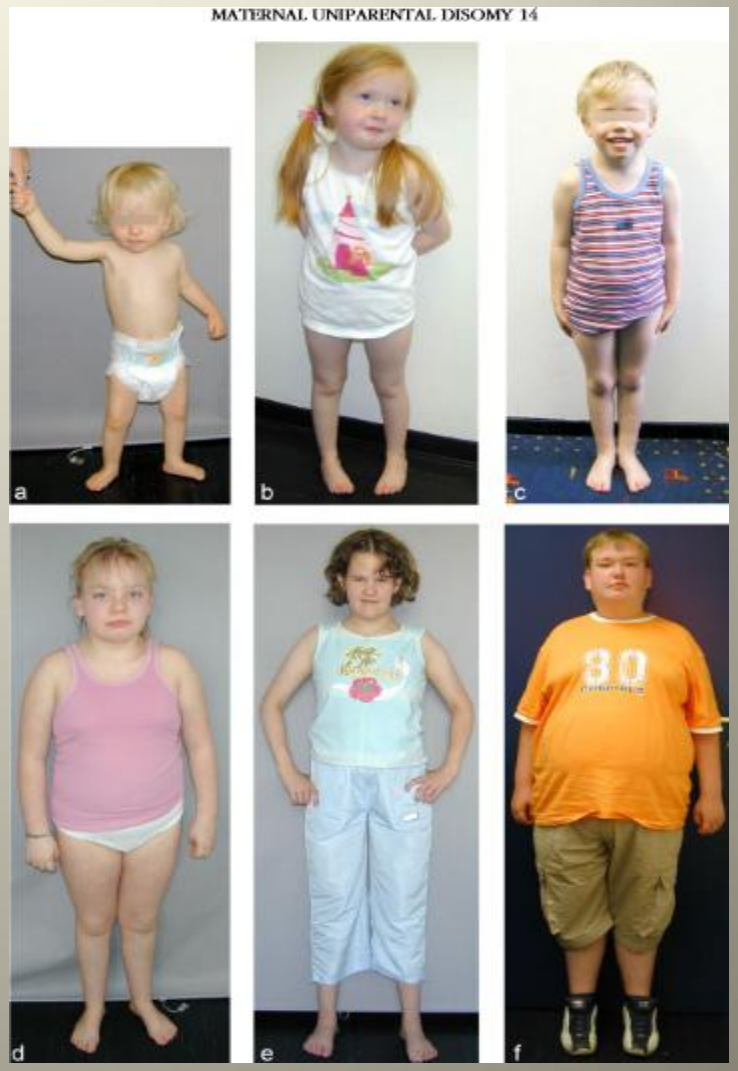
Case 5

- **Sanger sequencing revealed that a novel variant c.896C>A (p.S299Y) in the *RIPK3* (NM_006871, chr14:24806905, *14q12*, non disease associated gene) is homozygous in this individual, heterozygous in the mother and negative in the father. These data support maternal uniparental disomy (UPD) on chromosome 14 in this individual.**



UPD(14)mat Resembles PWS

	This Patient	UPD(14)mat (n=36)
IUGR	-	12/13
Low birth weight	20ile	18/21
Short statue	25-50ile	20/24
Obesity	90-95ile	10/15
Hypotonia	+	18/21
Feeding difficulties	+	9/25
Joint laxity	+	7/11
Facial dysmorphisms	+	16/20
Motor delay	+	17/25
Mental delay	Language delay	8/24
Premature puberty	Too young	11/12



Mitter et al, *Am J Med Genet A*, 2006

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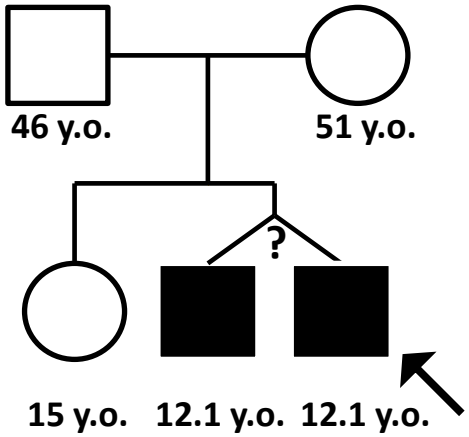
Mosaicism in a parent causing recurrent AD condition in the family

- **Clinical Presentation:**
  - Twin brother delayed speech, developmental regression, autism/autistic spectrum, intellectual disability, seizure disorder, short stature, microcephaly, dysmorphic features, and congenital heart disease.
- WES was requested on the proband only
- Samples from twin brother, unaffected sister and parents were available for Sanger studies



SHANK3 SH3 and multiple ankyrin repeat domains 3, c.3329_3332del (p.I1110fs), chr. 22q13.33.
Both parents are negative, the twin brother is also heterozygous, the unaffected sister is negative,
Associated disease: Phelan-McDermid syndrome [MIM:606232], **AD**

Blended Phenotypes with Two Diagnoses

Case	Disease I	Disease II
1	Ataxia-telangiectasia	Spastic paraplegia 50
2	Carpenter Syndrome	Neurofibromatosis, type 1
3	Nicolaides-Baraitser syndrome	Dravet syndrome
4	Contractural arachnodactyly, congenital	Renpenning syndrome
5	Epilepsy, progressive myoclonic 5	Rubinstein-Taybi syndrome
6	Leigh syndrome, X-linked	Bardet-Biedl syndrome 10
7	Mental retardation, autosomal dominant 12	Mental retardation, X-linked 94
8	Cardiomyopathy	Duchenne muscular dystrophy
9	Malformations of cortical development and microcephaly	Pitt-Hopkins-like syndrome 2
10	Rothmund-Thomson syndrome	Xeroderma pigmentosum, group C
11	Epilepsy, juvenile absence, susceptibility to, 1	Cornelia de Lange syndrome 2

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  - Explore all possible inheritance manners
  - Don’t stop at one diagnosis, the patient could have blended phenotypes resulting from two single gene defects
  - **Incorporating clinical expertise in exome reporting**
    - **Weekly WGL meeting**
    - **Monthly WES sign-out meeting: accessible worldwide from the internet**
- Building and sharing knowledge database
- New disease gene discoveries

Key Elements of Clinical Exome

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  - Incorporating clinical expertise in exome reporting
- **Building and sharing knowledge database**
  - **Data submission from WGL to ClinVar, etc.**
- **New disease gene discoveries**

New Gene Discoveries

REPORT

De Novo Truncating Mutations in *AHDC1* in Individuals with Syndromic Expressive Language Delay, Hypotonia, and Sleep Apnea

AJHG

REPORT

Mutations in *PURA* Cause Profound Neonatal Hypotonia, Seizures, and Encephalopathy in 5q31.3 Microdeletion Syndrome

AJHG

LETTERS

nature genetics

Bainbridge *et al. Genome Medicine* 2013, **5**:11
http://genomemedicine.com/content/5/2/11

Genome Medicine

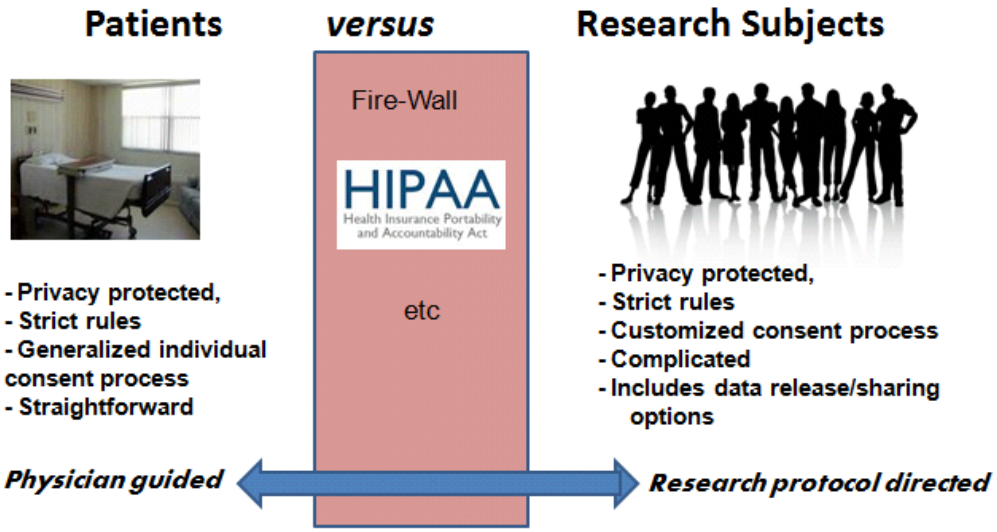
RESEARCH **Open Access**

De novo truncating mutations in *ASXL3* are associated with a novel clinical phenotype with similarities to Bohring-Opitz syndrome

...runcating mutations of *MAGEL2* cause Prader-Willi ...henotypes and autism

New Disease Gene Discoveries

- Opportunity for unsolved exome negative cases to join research studies



- Clinical variant data sharing: technically easy
- Obeying the rules: NOT TRIVIAL

Clinical lab beginning to be preferred pathway for sample recruitment!!!!

Global Collaborations are Essential

2nd IRDiRC Conference - Shenzhen

7 – 9 NOVEMBER, 2014 FUTIAN SHERATON HOTEL, SHENZHEN, CHINA

WHOLE GENOME LABORATORY

HUMAN GENOME SEQUENCING CENTER
Baylor College of Medicine

http://www.bcm.edu/geneticlabs/index.cfm?PMID=21319

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