Implementing clinical whole exome sequencing for the care of children with Mendelian disorders and cancer

Sharon E. Plon, MD, PhD, FACMG

Departments of Pediatrics/Hematology-Oncology and Molecular and Human Genetics

Human Genome Sequencing Center

Baylor College of Medicine

Baylor College of Medicine

HGSC
HUMAN GENOME SEQUENCING CENTER

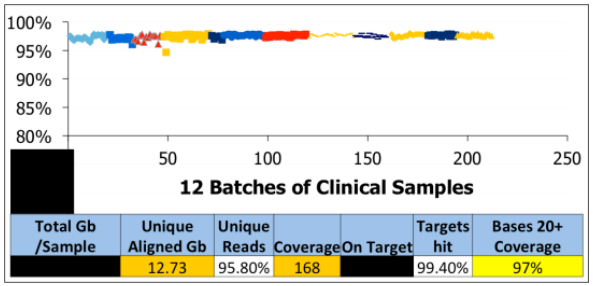
Texas Children's Cancer and Hematology Centers

Disclosures – Sharon E. Plon, MD, PhD

- **I have the following financial relationships to disclose:**
  - I am a member of the Baylor Genetics Scientific Advisory Board
- **I will not discuss specific off label use and/or investigational use in my presentation.**

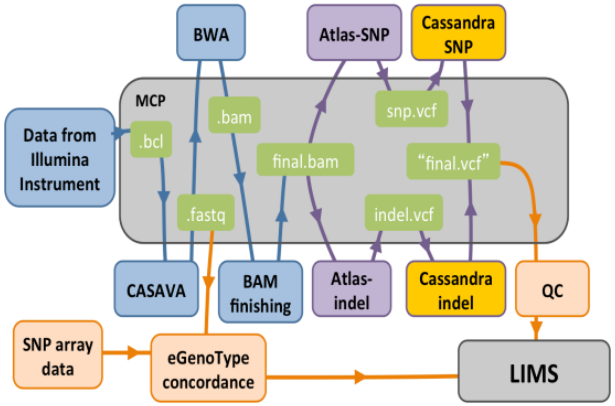
HGSC
HUMAN GENOME SEQUENCING CENTER

DNA/WES Technology



Total Gb /Sample	Unique Aligned Gb	Unique Reads	Coverage	On Target	Targets hit	Bases 20+ Coverage
	12.73	95.80%	168		99.40%	97%

Informatics



Process Management
Quality Control

WGL
WHOLE GENOME LABORATORY

MGL
MEDICAL GENETICS LABORATORIES

Dept of Molecular and Human Genetics

Physicians/Counselors
Business Management
Sales/Marketing
Web Communications
Sales\Follow up
Regulatory

cap ACCREDITED™

CMS
CENTERS for MEDICARE & MEDICAID SERVICES

AMERICAN BOARD OF MEDICAL GENETICS

Clinical Exome Sequencing Timeline

2011
Clinical exome sequencing

2012
NHGRI CSER U01

2013
ACMG IF guidelines

2013
NEJM 250 Cases

Yaping Yang

ORIGINAL ARTICLE

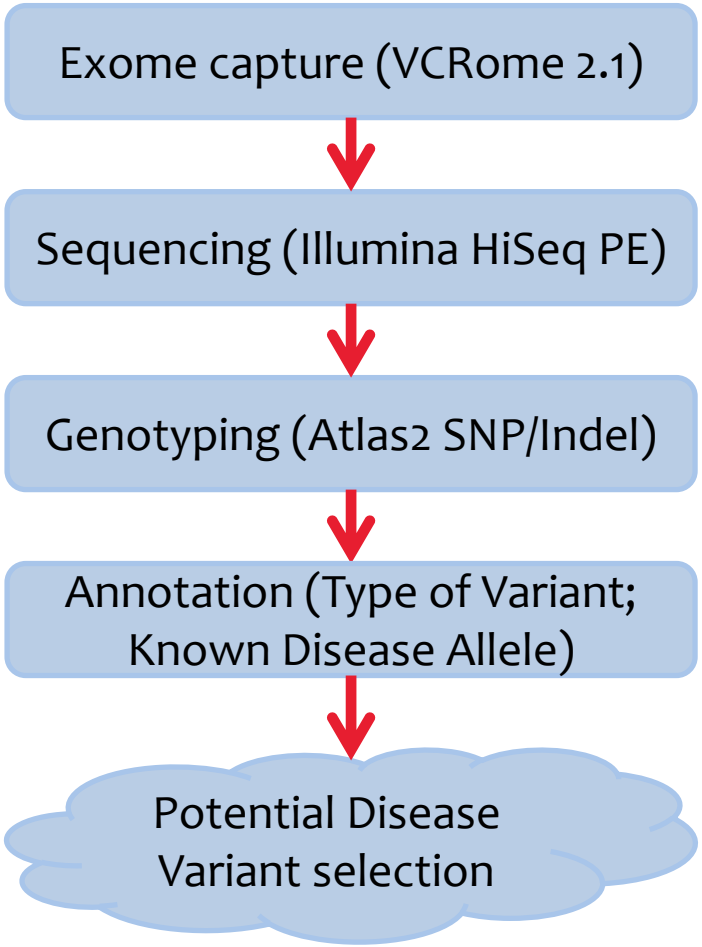
Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

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.

Christine Eng

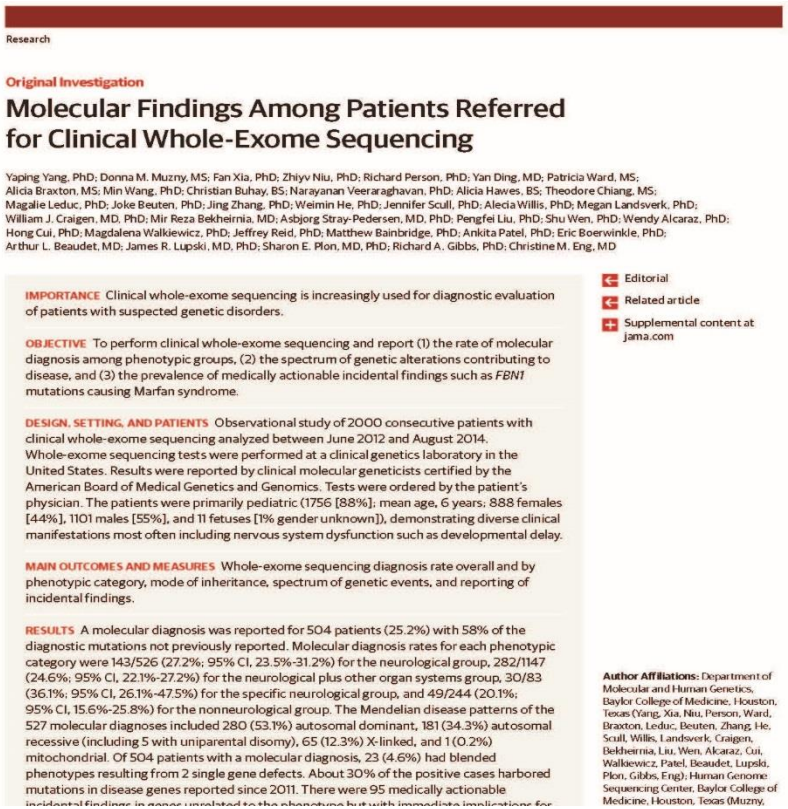
Analysis of Next Gen Data for Clinical Reporting

Analysis focuses on genes with *rare*, *protein-altering* changes *with appropriate mechanism of inheritance, in genes associated with disease*.



- *Rare:* given the severity of the phenotypes, the allele should not be present at polymorphism frequency (1%) in control populations
- *Protein-altering:* most likely to have biological consequence (especially loss of function mutations)
- *Disease genes:* is this variant in a gene known to be associated with Mendelian disease (OMIM, Pubmed)
- What is known about this particular variant (HGMD, ClinVar)
- ACMG/AMP Guideline for Variant Interpretation (Richards *GIM*, 2015)

Yang et al., *JAMA*, 2014 – Description of 2000 WES clinical cases



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

IMPORTANCE Clinical whole-exome sequencing is increasingly used for diagnostic evaluation of patients with suspected genetic disorders.

OBJECTIVE To perform clinical whole-exome sequencing and report (1) the rate of molecular diagnosis among phenotypic groups, (2) the spectrum of genetic alterations contributing to disease, and (3) the prevalence of medically actionable incidental findings such as *FBN1* mutations causing Marfan syndrome.

DESIGN, SETTING, AND PATIENTS Observational study of 2000 consecutive patients with clinical whole-exome sequencing analyzed between June 2012 and August 2014. Whole-exome sequencing tests were performed at a clinical genetics laboratory in the United States. Results were reported by clinical molecular geneticists certified by the American Board of Medical Genetics and Genomics. Tests were ordered by the patient's physician. The patients were primarily pediatric (1756 [88%]; mean age, 6 years; 888 females [44%], 1101 males [55%], and 11 fetuses [1% gender unknown]), demonstrating diverse clinical manifestations most often including nervous system dysfunction such as developmental delay.

MAIN OUTCOMES AND MEASURES Whole-exome sequencing diagnosis rate overall and by phenotypic category, mode of inheritance, spectrum of genetic events, and reporting of incidental findings.

RESULTS A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. Molecular diagnosis rates for each phenotypic category were 143/526 (27.2%; 95% CI, 23.5%-31.2%) for the neurological group, 282/1147 (24.6%; 95% CI, 22.1%-27.2%) for the neurological plus other organ systems group, 30/83 (36.1%; 95% CI, 26.1%-47.5%) for the specific neurological group, and 49/244 (20.1%; 95% CI, 15.6%-25.8%) for the nonneurological group. The Mendelian disease patterns of the 527 molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal recessive (including 5 with uniparental disomy), 65 (12.3%) X-linked, and 1 (0.2%) mitochondrial. Of 504 patients with a molecular diagnosis, 23 (4.6%) had blended phenotypes resulting from 2 single gene defects. About 30% of the positive cases harbored mutations in disease genes reported since 2011. There were 95 medically actionable

Editorial

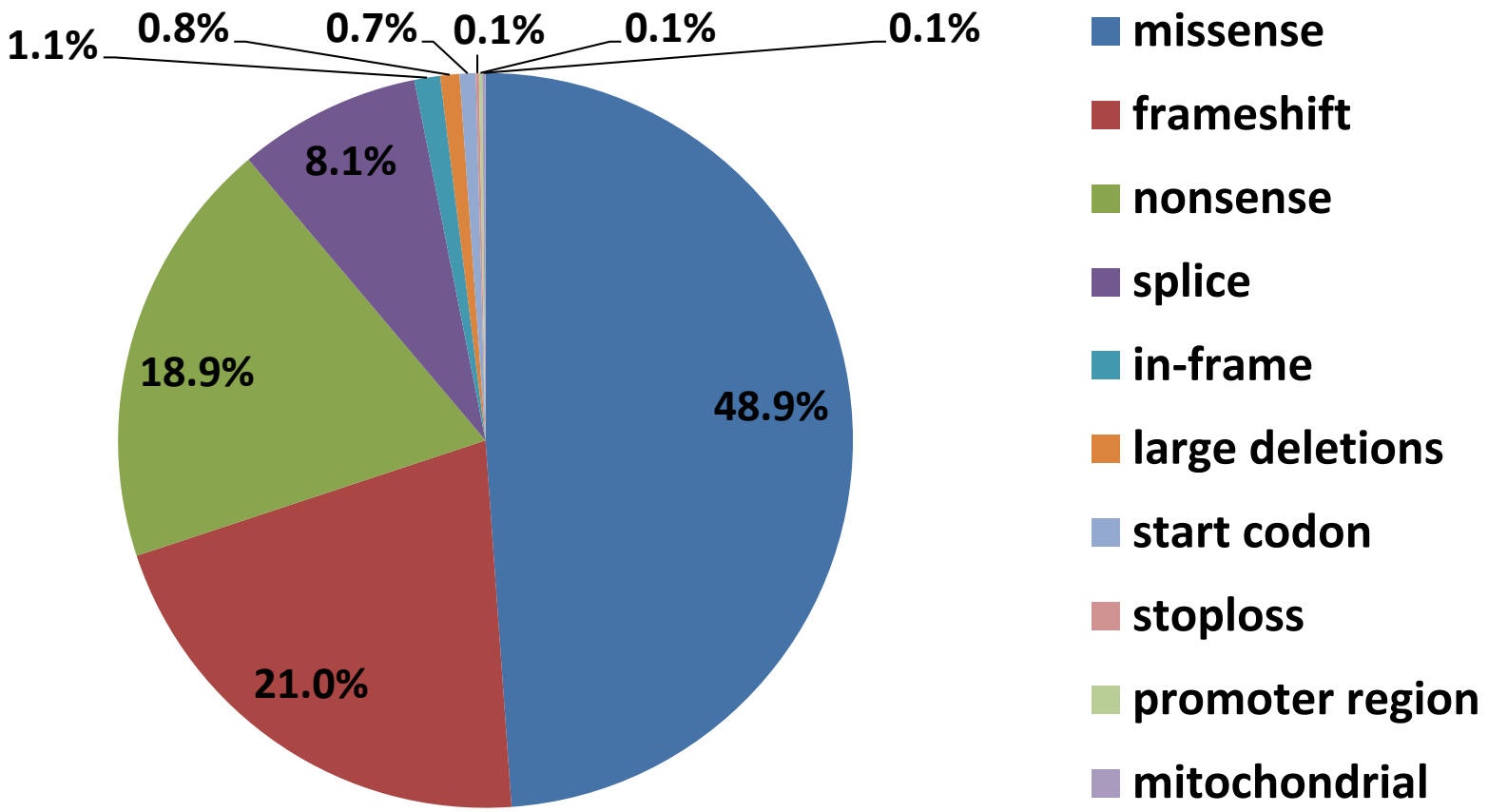
Related article

Supplemental content at jama.com

Author Affiliations: Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas (Yang, Xia, Niu, Person, Ward, Braxton, Leduc, Beuten, Zhang, He, Scull, Willis, Landsverk, Craigen, Bekheirnia, Liu, Wen, Alcaraz, Cui, Walkiewicz, Patel, Beaudet, Lupski, Plon, Gibbs, Eng); Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas (Muzny,

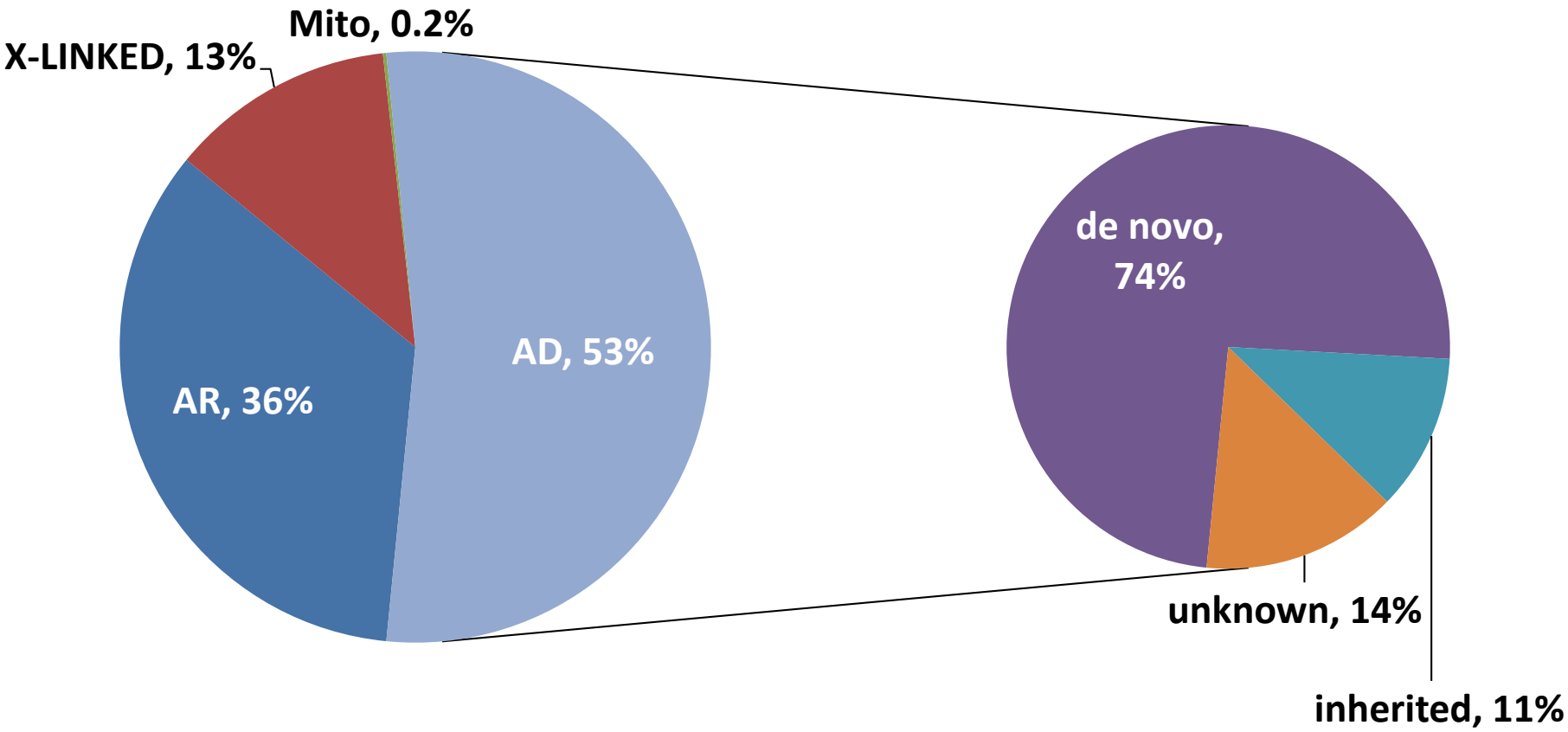
- 1780 predominantly pediatric patients (89%)
- 1440 (72%) have intellectual disability, seizure disorder or autism
- **Diagnostic rate ~25% for patients referred for proband only WES.**
- Now completed over 12,000 clinical cases

Mutations in Positive WES Cases



708 Mutant Alleles in the 504 Positives, 409 (58%) novel at time of reporting

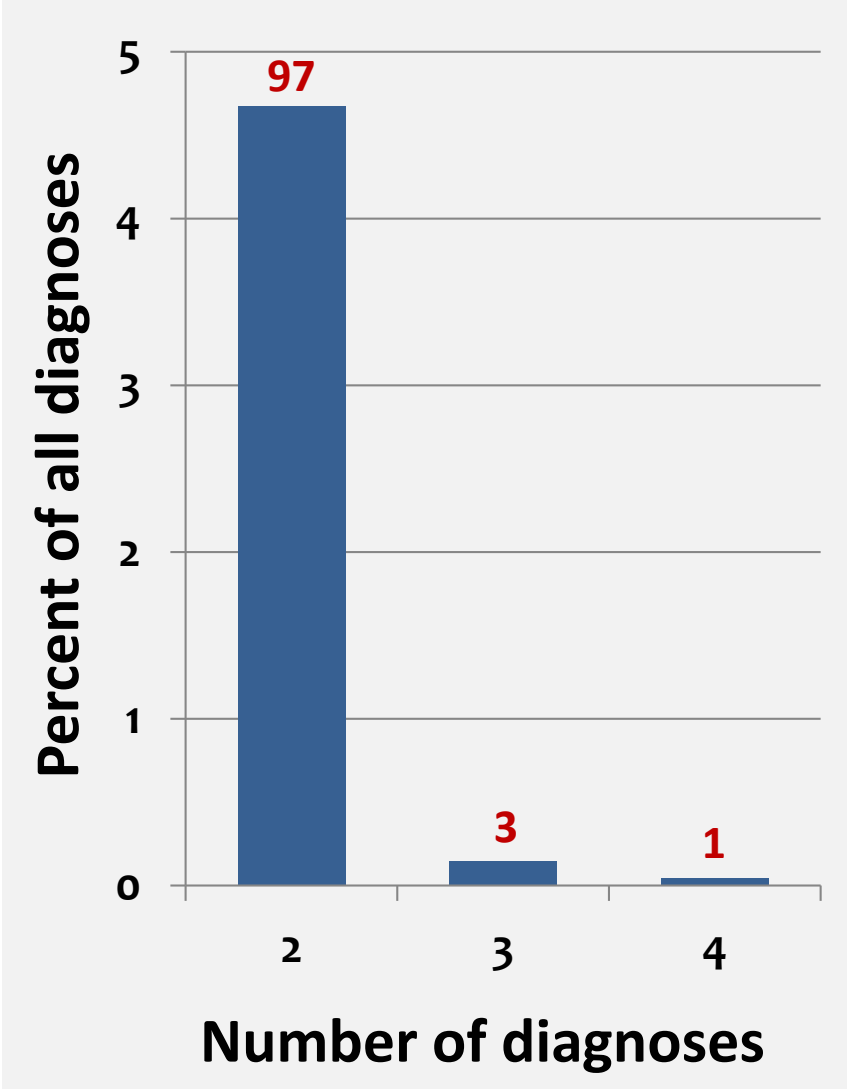
Most Mutant Alleles Arose de Novo (AD: 74%; XL: 62%)



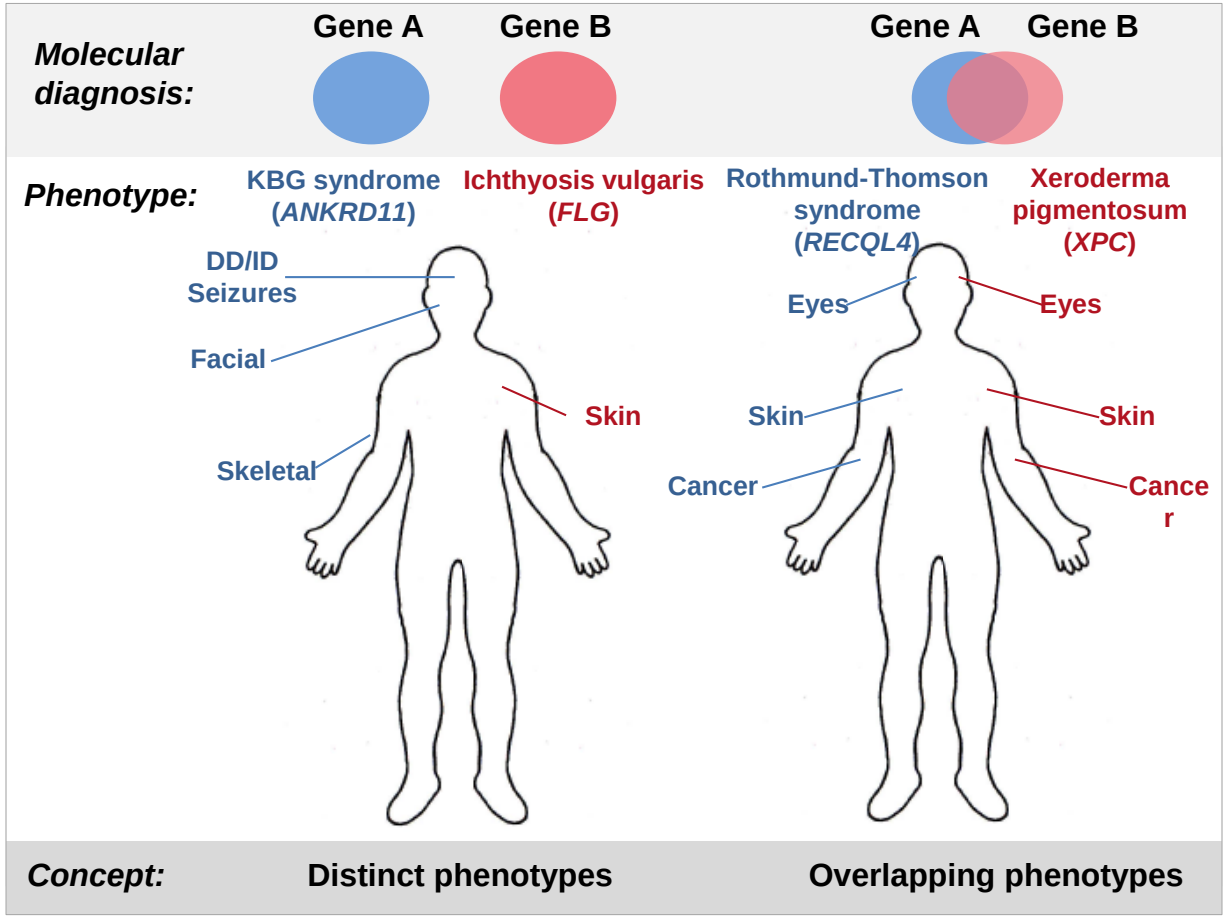
Multiple Mendelian Diagnoses in WES Cases

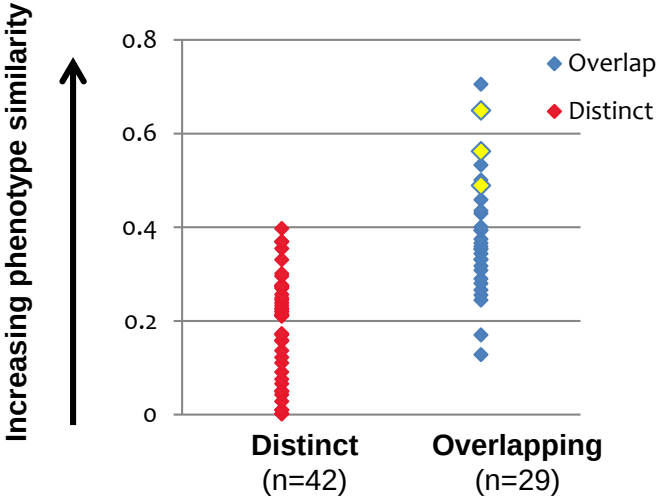
7374 sequential cases submitted for proband WES

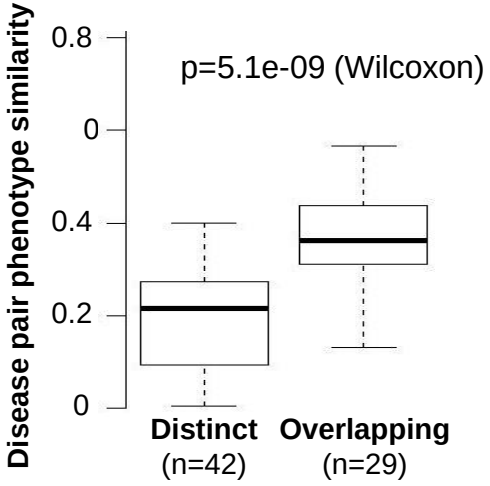
- Diagnosis in **28.2%** (2076/7374)
- Two or more diagnoses **related to phenotype** in **4.9%** (101/2076) of diagnosed cases



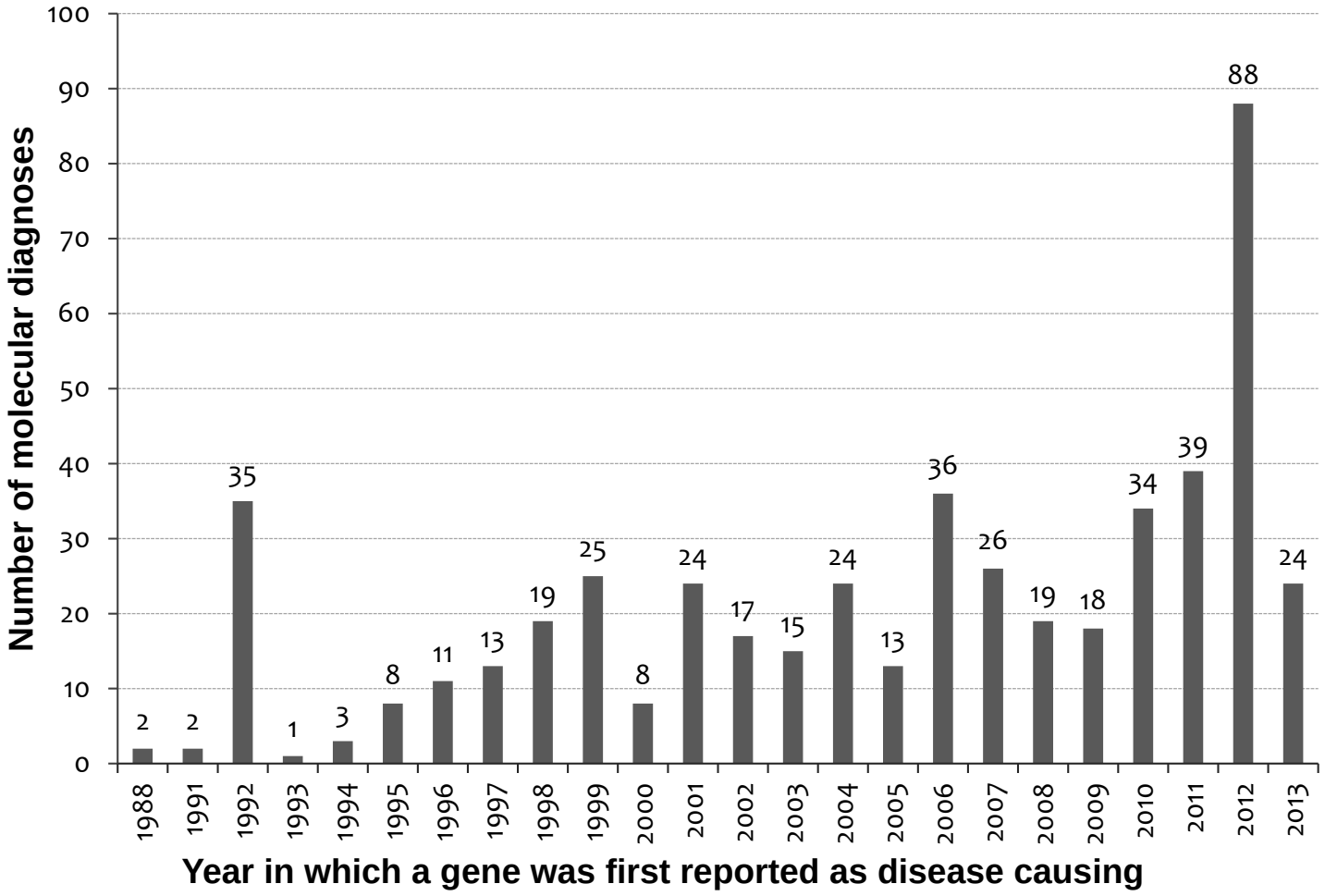
Posey et al, *NEJM,* 2017


Molecular diagnosis:
Gene A
Gene B
Gene A
Gene B
Phenotype:
KBG syndrome (ANKRD11)
Ichthyosis vulgaris (FLG)
Rothmund-Thomson syndrome (RECQL4)
Xeroderma pigmentosum (XPC)
DD/ID
Seizures
Facial
Skeletal
Skin
Eyes
Skin
Cancer
Eyes
Skin
Cancer
Concept:
Distinct phenotypes
Overlapping phenotypes


Increasing phenotype similarity
0.8
0.6
0.4
0.2
0
Overlap
Distinct
Distinct
(n=42)
Overlapping
(n=29)

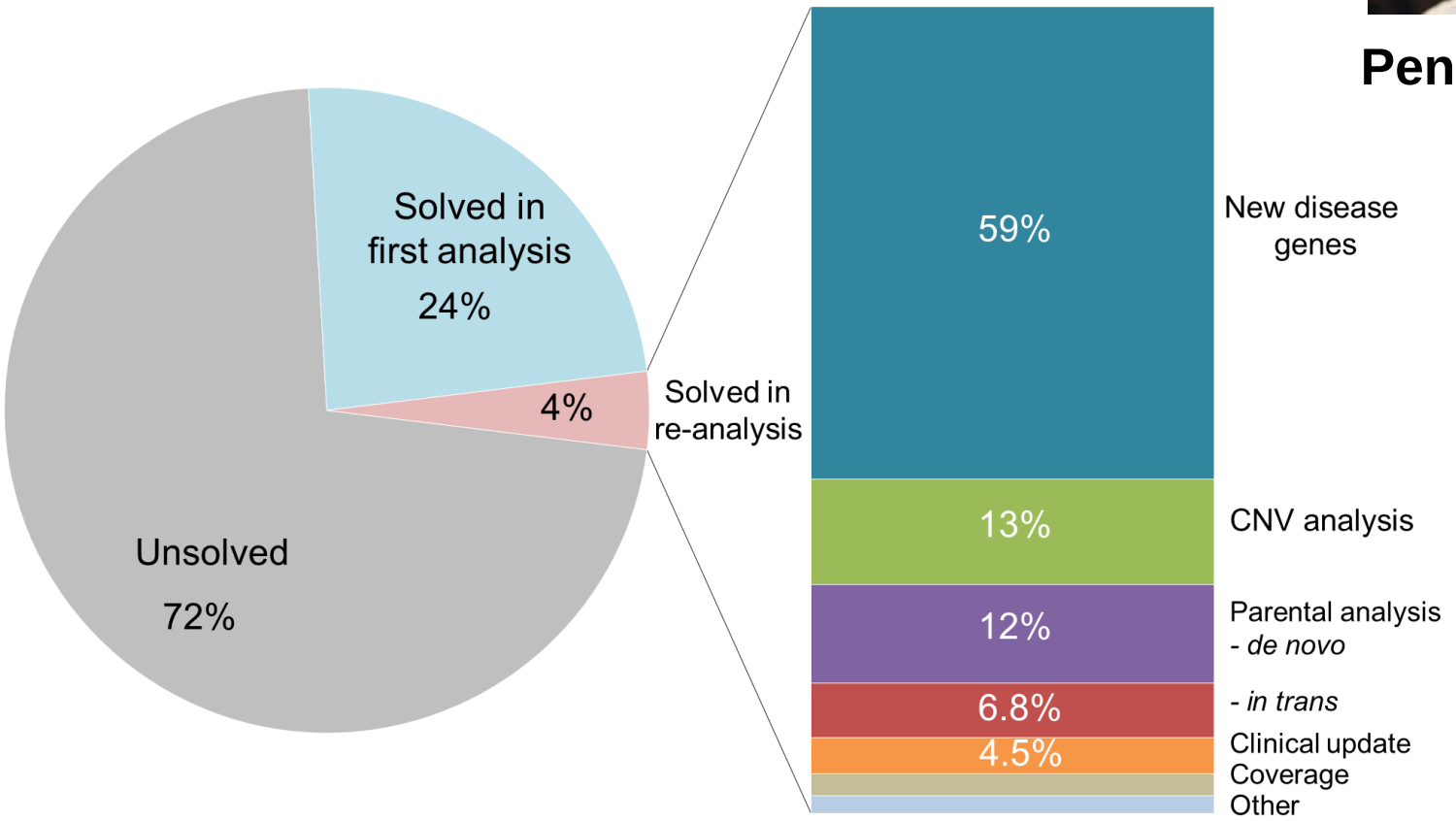

Disease pair phenotype similarity
0.8
0
0.4
0.2
0
p=5.1e-09 (Wilcoxon)
Distinct
(n=42)
Overlapping
(n=29)

Diagnostic rate heavily dependent on newly discovered disease genes



WES re-analysis increases diagnostic rate over time

Pengfei Liu



Discovery of new disease genes is the greatest contributor to improved diagnostic rate

# of patients solved after re-analysis	Name of new disease genes
>5	*DDX3X, PURA, TANGO2*, KAT6A, PIK3R1*
3~5	*SLC1A4, DNM1, POZ, AHDC1, ARID2, ECHS1, GNAO1, KCNA2, MAGEL2, SLC13A5, SOX5, WDR73*
1~2	*ASXL3, CHAMP1, CHD8, DEPDC5, HNRNPU, KCNT1, NALCN, PPP2R5D, PUF60, VARS2, WDR45, ADNP, CNTNAP1, DNM1L, FBXL4, KCNC1, KMT2A, LAS1L, LIPT1, LZTR1, MED13L, MLL, NR2F1, PMPCA, RAB3GAP2, RARB, SERAC1, SSR4, STAMBP, VRK1, ZBTB20*

JAMA Pediatrics | Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Use of Exome Sequencing for Infants in Intensive Care Units
Ascertainment of Severe Single-Gene Disorders and Effect on Medical Management

Linyan Meng, PhD; Mohan Pammi, MD, PhD; Anirudh Saronwala, MD; Pilar Magoulas, MS; Andrew Ray Ghazi, BS; Francesco Vetrini, PhD; Jing Zhang, PhD; Weimin He, PhD; Avinash V. Dharmadhikari, PhD; Chunjing Qu, PhD; Patricia Ward, MS; Alicia Braxton, MS; Swetha Narayanan, MS; Xiaoyan Ge, PhD; Mari J. Tokita, MD; Teresa Santiago-Sim, PhD; Hongzheng Dai, PhD; Theodore Chiang, MSc; Hadley Smith, MPSA; Mahshid S. Azamian, MD, MPH; Laurie Robak, MD, PhD; Bret L. Bostwick, MD; Christian P. Schaaf, MD, PhD; Lorraine Potocki, MD; Fernando Scaglia, MD; Carlos A. Bacino, MD; Neil A. Hanchard, MD, PhD; Michael F. Wangler, MD; Daryl Scott, MD, PhD; Chester Brown, MD; Jianhong Hu, PhD; John W. Belmont, MD, PhD; Lindsay C. Burrage, MD, PhD; Brett H. Graham, MD; Vernon Reid Sutton, MD; William J. Craigen, MD, PhD; Sharon E. Plon, MD, PhD; James R. Lupski, MD, PhD, DSc(hon); Arthur L. Beaudet, MD; Richard A. Gibbs, PhD; Donna M. Muzny, MS; Marcus J. Miller, PhD; Xia Wang, PhD; Magalie S. Leduc, PhD; Rui Xiao, PhD; Pengfei Liu, PhD; Chad Shaw, PhD; Magdalena Walkiewicz, PhD; Weimin Bi, PhD; Fan Xia, PhD; Brendan Lee, MD, PhD; Christine M. Eng, MD; Yaping Yang, PhD; Seema R. Lalani, MD

Results of WES testing for 278 critically ill infants <100 days

- Overall 36.7% received a genetic diagnosis.
- Critical trio (14 day TAT) had a higher yield with 32 of 63 infants achieving diagnosis (50.8%).
- Diagnostic rate lower in children with cardiovascular disorders.
- Medical management was affected for 52.0% with diagnoses. These included:
  - Changing care or adding needed diagnostic testing.
  - Withdrawal of care in children with lethal diagnoses

Critical Trio Example

- Clinical presentation:
  - 4-day-old male
  - IUGR, admitted to NICU due to respiratory distress, pale skin, petechiae and bruising on chest and back
- Initial lab work revealed pancytopenia
- Critical trio WES (TAT 10d):
  - FANCA, c.154C>T (p.R52X), c.2852G>A, p.R951Q, both pathogenic, compound heterozygous
- Fanconi anemia, complementation group A [MIM: 227650]

Newborn diagnosis of Fanconi Anemia

- Represents an extraordinarily early presentation of FA
  - Average age of bone marrow failure – 6 years
  - Only a few other case reports of newborn presentation
- Clinical management after WES:
  - Postpone bone marrow biopsy
  - Early plan for bone marrow transplantation
  - Monitoring for other systems: renal ultrasound, echocardiogram
  - Early discharge and close follow up in clinic

Clinical Sequencing Exploratory Research

*Moving the **Genome** Into the **Clinic***

377 Researchers
21 Institutions
1 Consortium

cser
Clinical Sequencing Exploratory Research

www.genome.gov/CSER
www.cser-consortium.org

Baylor College of Medicine BASIC3 Key Team Members

Sharon Plon

Murali Chintagumpala

Stacey Berg

Richard Gibbs

Christine Eng

David Wheeler

Sue Hilsenbeck

Laurence McCullough

Richard Street

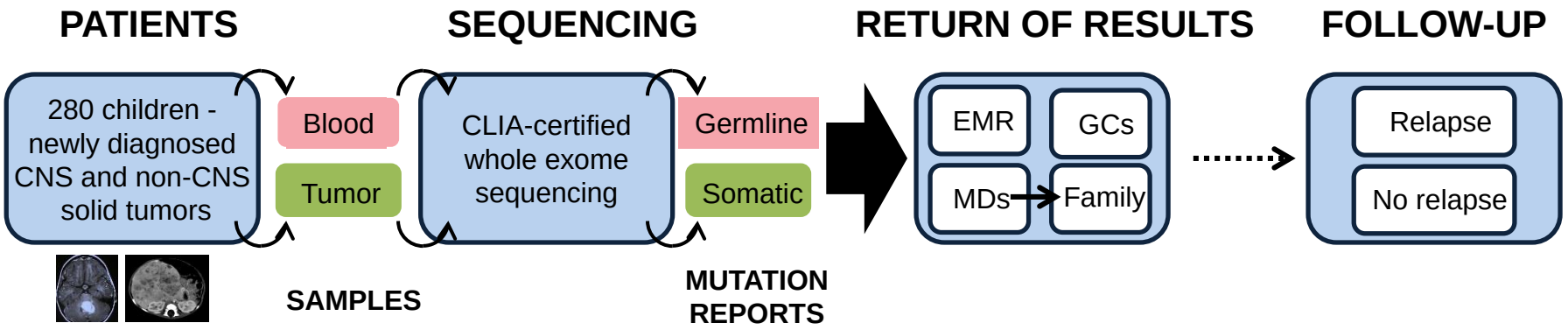
Amy McGuire

Angshumoy Roy

Dolores Lopez-Terrada

BASIC^3

Baylor College of Medicine **A**dvancing **S**equencing **I**nto **C**hildhood **C**ancer **C**are



Study objectives:

- To integrate information from CLIA-certified germline and tumor exome sequencing into the care of newly diagnosed solid and brain tumor patients at Texas Children's Cancer Center
- To perform parallel evaluation of the impact of tumor and germline exomes on families and physicians

Will Parsons
Pediatric Oncology

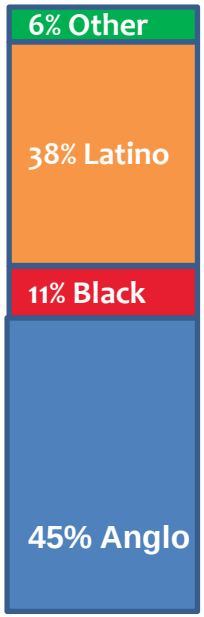
Original Investigation

Diagnostic Yield of Clinical Tumor and Germline Whole-Exome Sequencing for Children With Solid Tumors

D. Williams Parsons, MD, PhD; Angshumoy Roy, MD, PhD; Yaping Yang, PhD; Tao Wang, PhD; Sarah Scollon, MS, CGC; Katie Bergstrom, MS, CGC; Robin A. Kerstein, BS, MT; Stephanie Gutierrez, BS; Andrea K. Petersen, MD; Abhishek Bavle, MD; Frank Y. Lin, MD; Dolores H. López-Terrada, MD, PhD; Federico A. Monzon, MD; M. John Hicks, MD, PhD, DDS; Karen W. Eldin, MD; Norma M. Quintanilla, MD; Adekunle M. Adesina, MD, PhD; Carrie A. Mohila, MD, PhD; William Whitehead, MD; Andrew Jea, MD; Sanjeev A. Vasudevan, MD; Jed G. Nuchtern, MD; Uma Ramamurthy, PhD; Amy L. McGuire, JD, PhD; Susan G. Hilsenbeck, PhD; Jeffrey G. Reid, PhD; Donna M. Muzny, MSc; David A. Wheeler, PhD; Stacey L. Berg, MD; Murali M. Chintagumpala, MD; Christine M. Eng, MD; Richard A. Gibbs, PhD; Sharon E. Plon, MD, PhD

JAMA Oncol. doi:10.1001/jamaoncol.2015.5699
Published online January 28, 2016.

Race/Ethnicity of BASIC3 Subjects are Representative of Houston Population



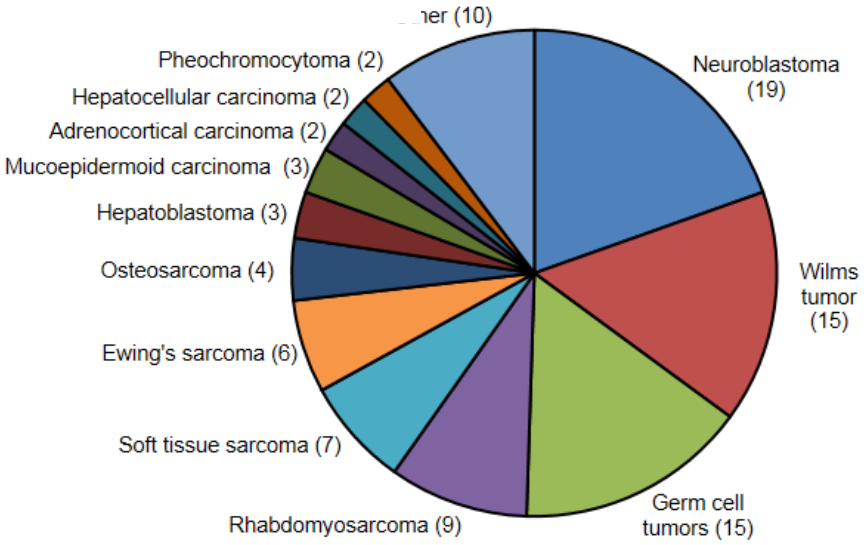
Characteristics of patients enrolled and not enrolled on study - updated

Characteristic - no. (%)	Enrolled (n=239)	Declined (n=103)	P Value
Ethnicity			0.54
Hispanic	111 (46%)	41 (40%)	
Non-Hispanic	119 (50%)	52 (50%)	
Not reported	10 (4%)	10 (10%)	
Race			0.11
White	141 (59%)	74 (72%)	
Black or African American	25 (10%)	12 (12%)	
Asian	7 (3%)	4 (4%)	
American Indian or Alaska Native	10 (4%)	2 (2%)	
Multiple	14 (6%)	---	
Not reported	42 (18%)		

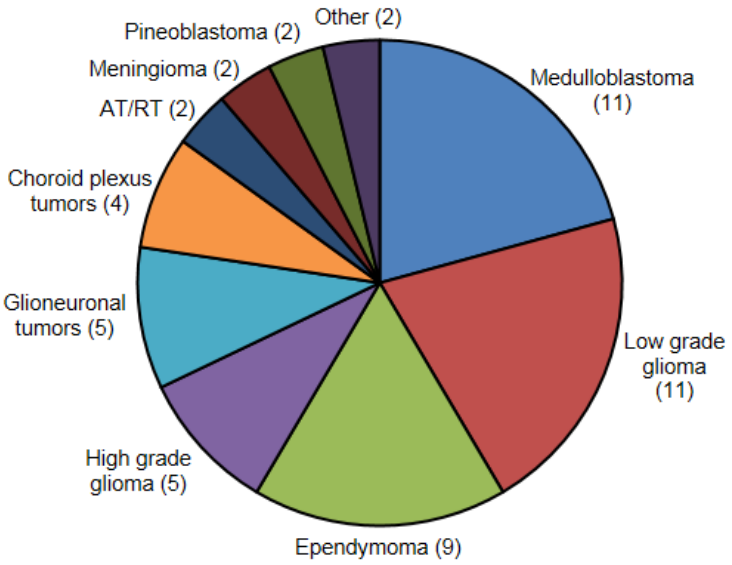
Updated from Scollon et al., *Genome Medicine* 2014

BASIC3 DIVERSE PEDIATRIC TUMOR DIAGNOSES

NON-CNS



CNS



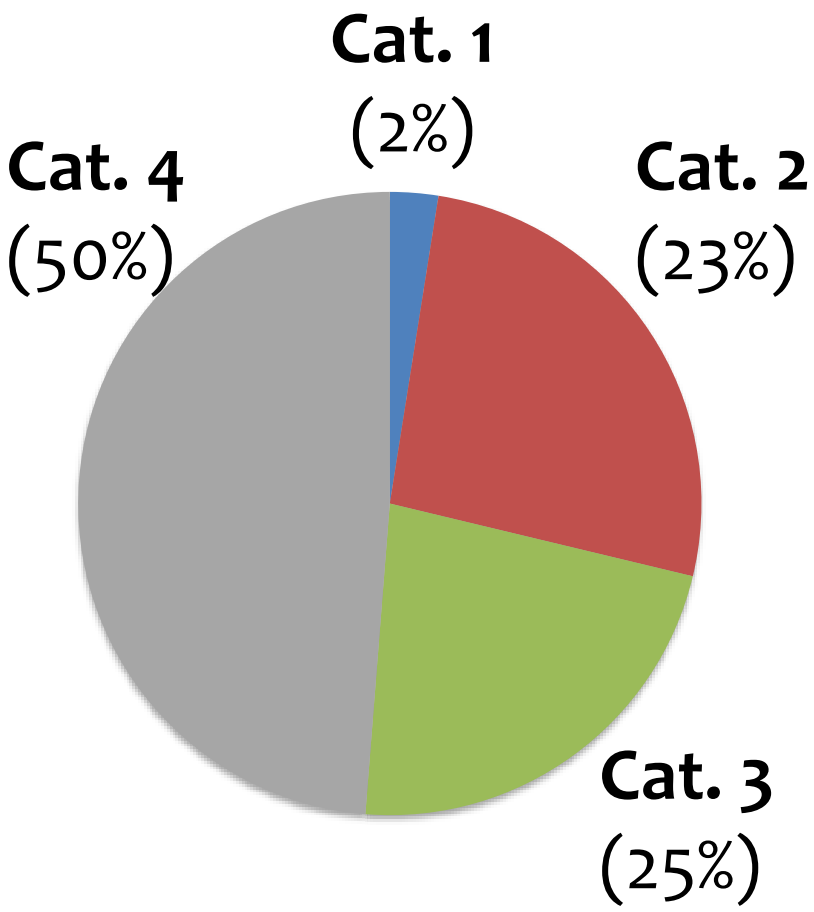
81/94 (86%)

40/56 (71%)

Tumor available for WES

Tumor WES Results (n=230)

HIGHEST category of mutation PER PATIENT



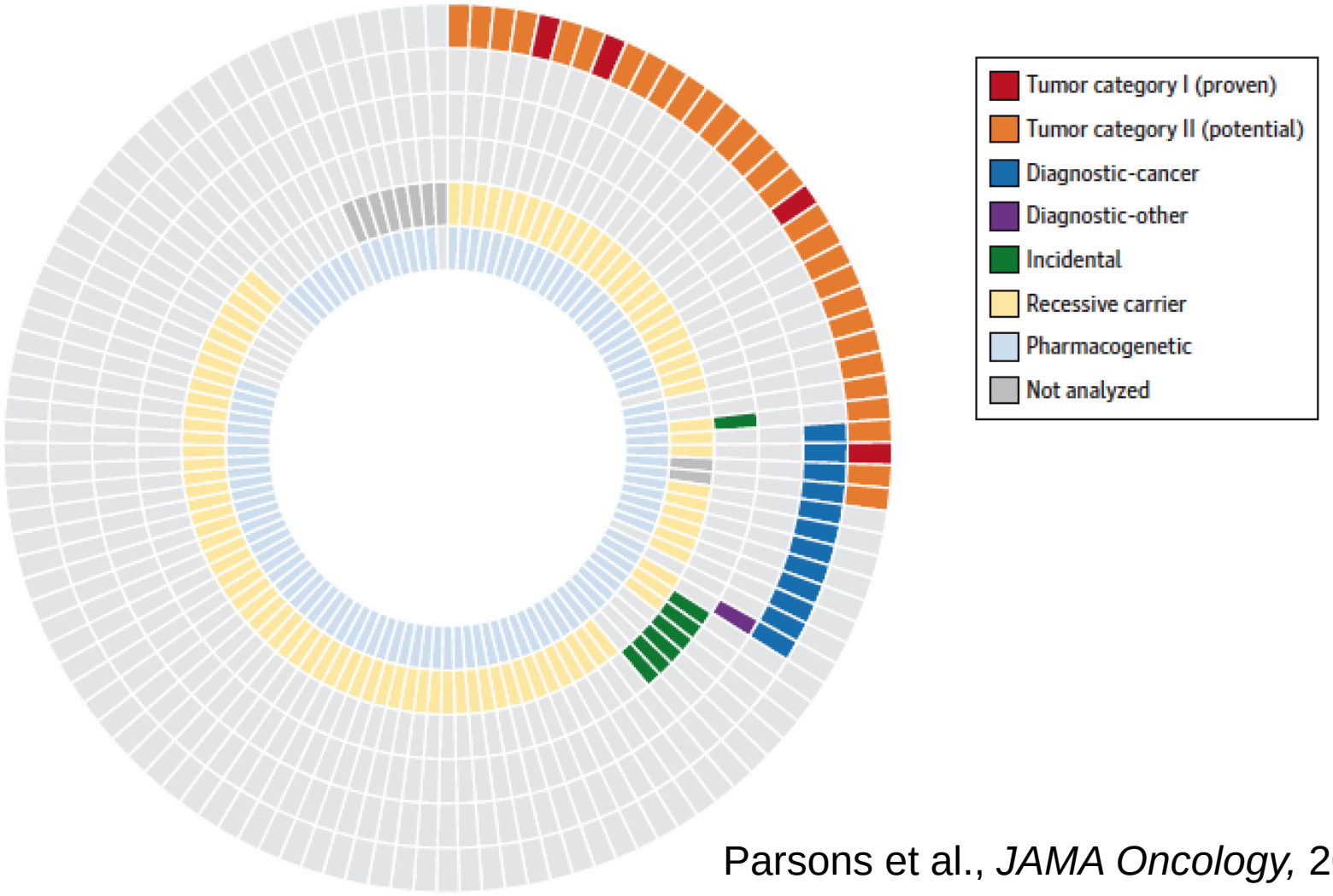
Categories of somatic mutations

- **I. Established clinical utility in tumor type**
  Example: *ALK* p.F1174L mutation (neuroblastoma)
- **II. Potential clinical utility**
  Example: *KRAS* p.Q61K mutation (neuroblastoma)
- **III. Consensus cancer genes**
  Example: *PHF6* mutation (neuroblastoma)
- **IV. Other genes**
  Example: *XIAP* mutation (neuroblastoma)

Now converting to new AMP variant curation

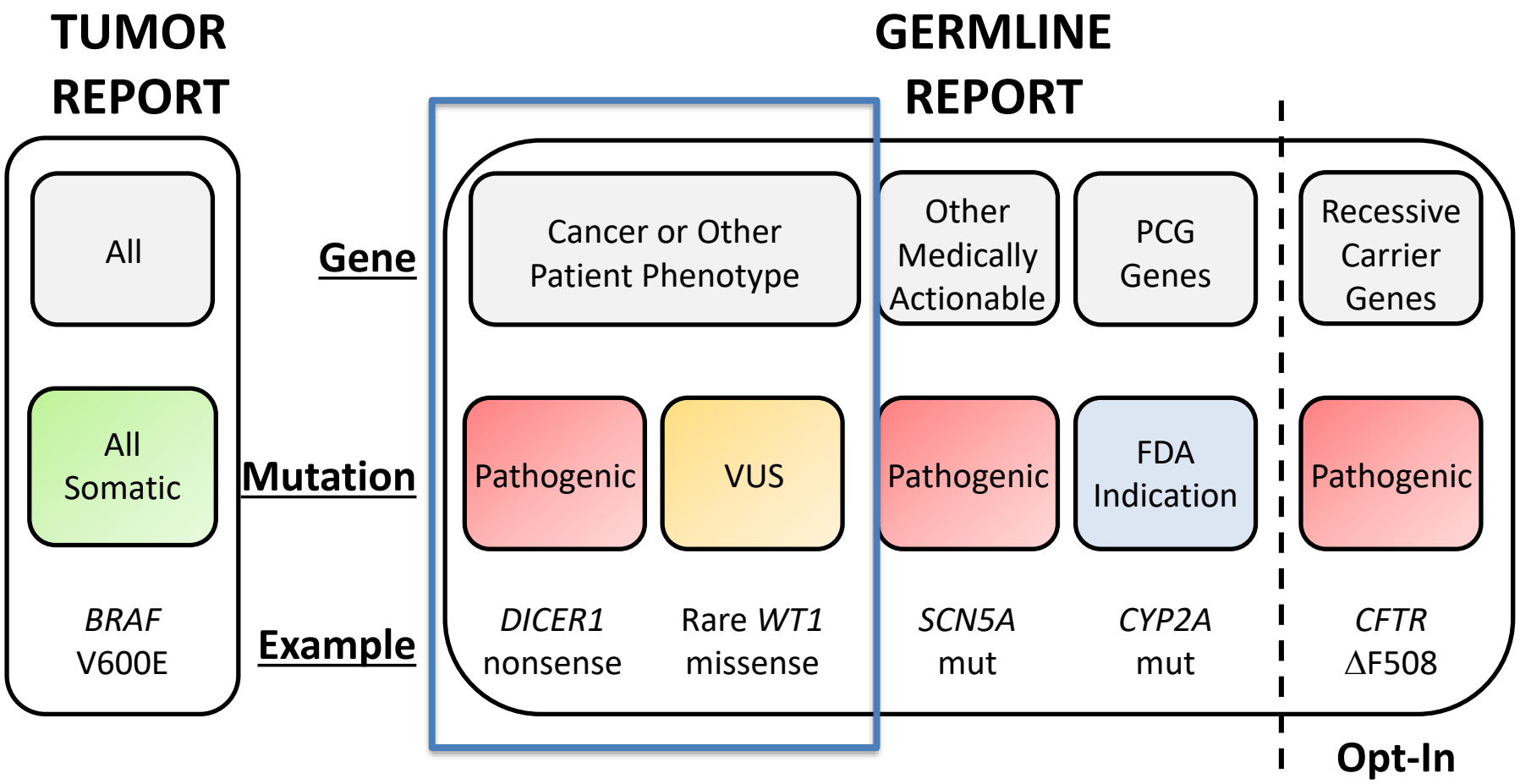
Germline and/or somatic mutations with potential clinical relevance found in 40% of cases

Figure 3. Combined Yield of Tumor and Germline Whole-Exome Sequencing (WES)



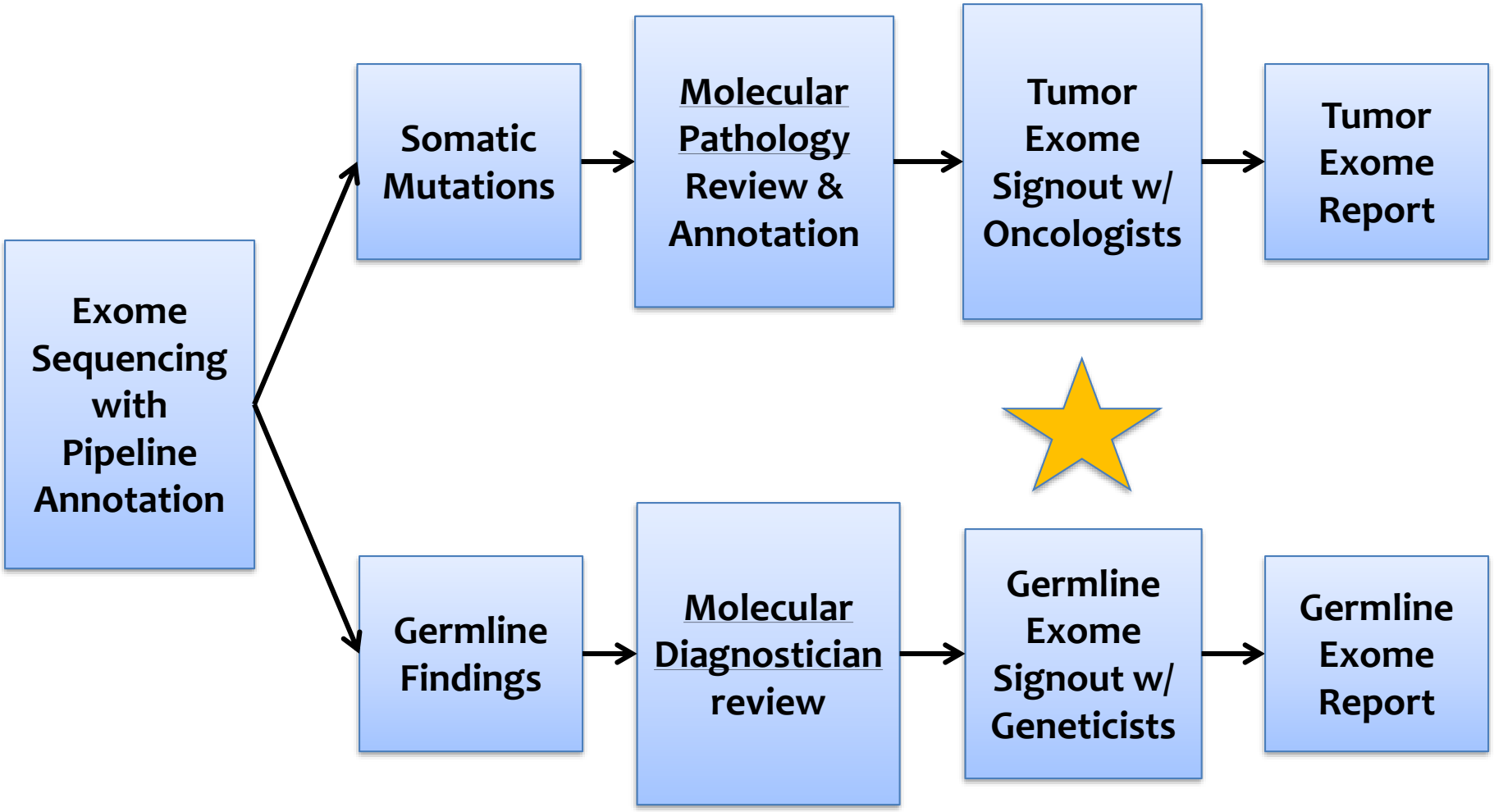
Parsons et al., *JAMA Oncology,* 2016

Diversity of germline results returned



Scollon et al., *Genome Medicine,* 2014

Two Exome Reporting Teams Work in Parallel



Variants of Uncertain "Clinical" Significance (VUS)

- Predominantly missense mutations in protein regions with or without known function.
- A variety of approaches including conservation, computational predictions, segregation with cancer and population studies are utilized to try and determine the significance.
- Different laboratories may report out same variant as a VUS or likely pathogenic or likely benign based on their laboratory's criteria.
  - Data sharing through ClinVar and other databases helps to decrease discordance across laboratories.

Ghosh *et al. Genome Biology* (2017) 18:225
DOI 10.1186/s13059-017-1353-5

Genome Biology

RESEARCH Open Access

Evaluation of *in silico* algorithms for use with ACMG/AMP clinical variant interpretation guidelines

Rajarshi Ghosh[1,2], Ninad Oak[1,2] and Sharon E. Plon[1,2*]

Significant discordance of missense predications across algorithms in current use

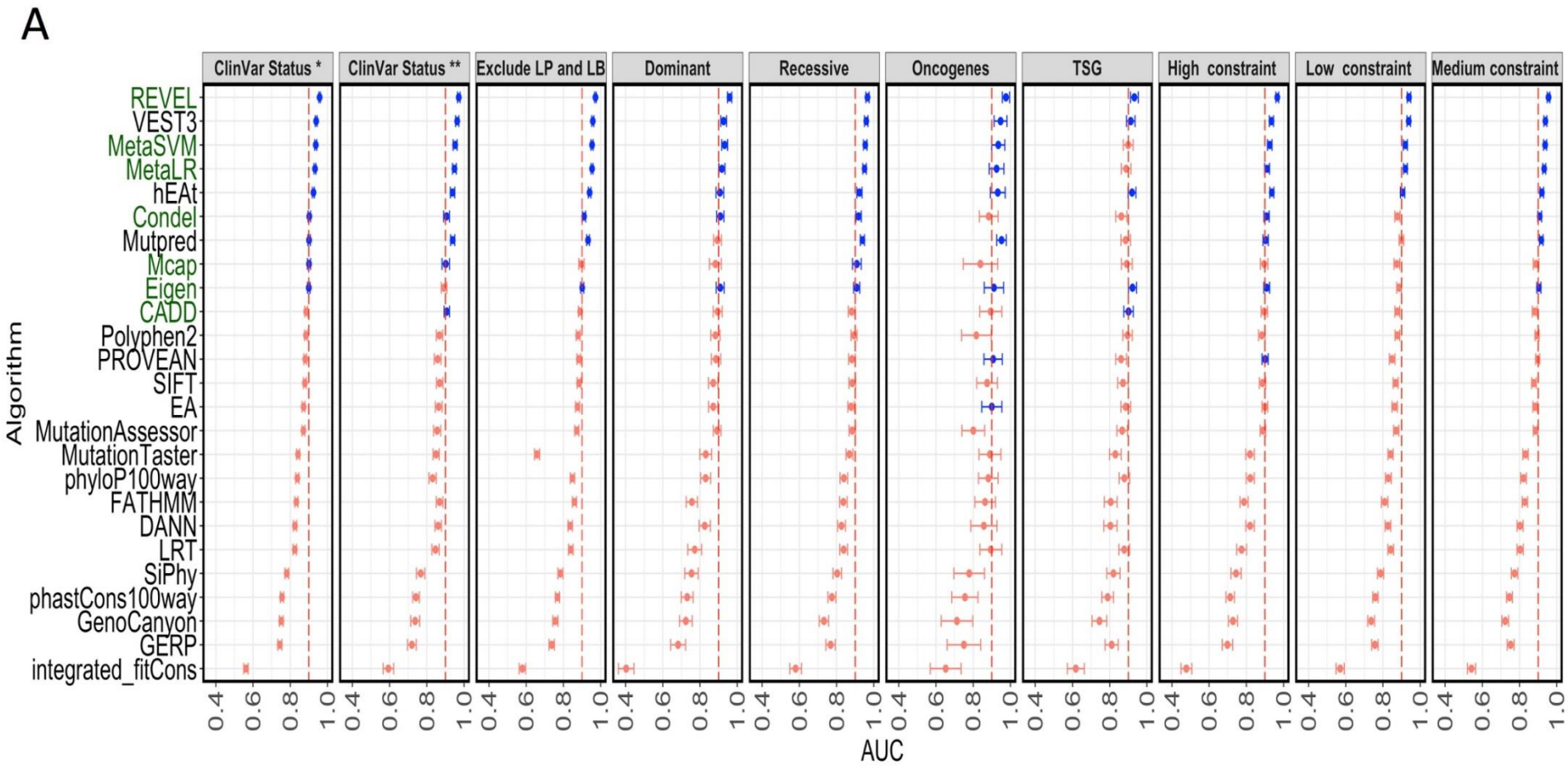
Table 1 Concordance rate of different combination of algorithms

Variant assertion in ClinVar	Variant source	Algorithms	Variants (n)	Concordance (*n* (%))	False concordance (n (%))
Benign	ClinVar*	All 18	7346	382 (5.2)	57 (0.8)
Pathogenic	ClinVar*	All 18	7473	2930 (39.2)	2 (0.03)
Benign	ClinVar**	All 18	1914	86 (4.5)	12 (0.6)
Pathogenic	ClinVar**	All 18	1052	492 (46.8)	0 (0)
Benign	ClinVar*	Polyphen, SIFT, CADD, PROVEAN, MutationTaster	7346	2464 (33.5)	815 (11.1)
Pathogenic	ClinVar*	Polyphen, SIFT, CADD, PROVEAN, MutationTaster	7473	5904 (79.0)	68 (0.9)
Benign	ClinVar*	Polyphen, SIFT, CADD	7346	3392 (46.2)	1340 (18.2)
Pathogenic	ClinVar*	Polyphen, SIFT, CADD	7473	6342 (84.9)	156 (2.1)

ClinVar *: ClinVar variants with one star or above review status
ClinVar **: ClinVar variants with two stars or above review status

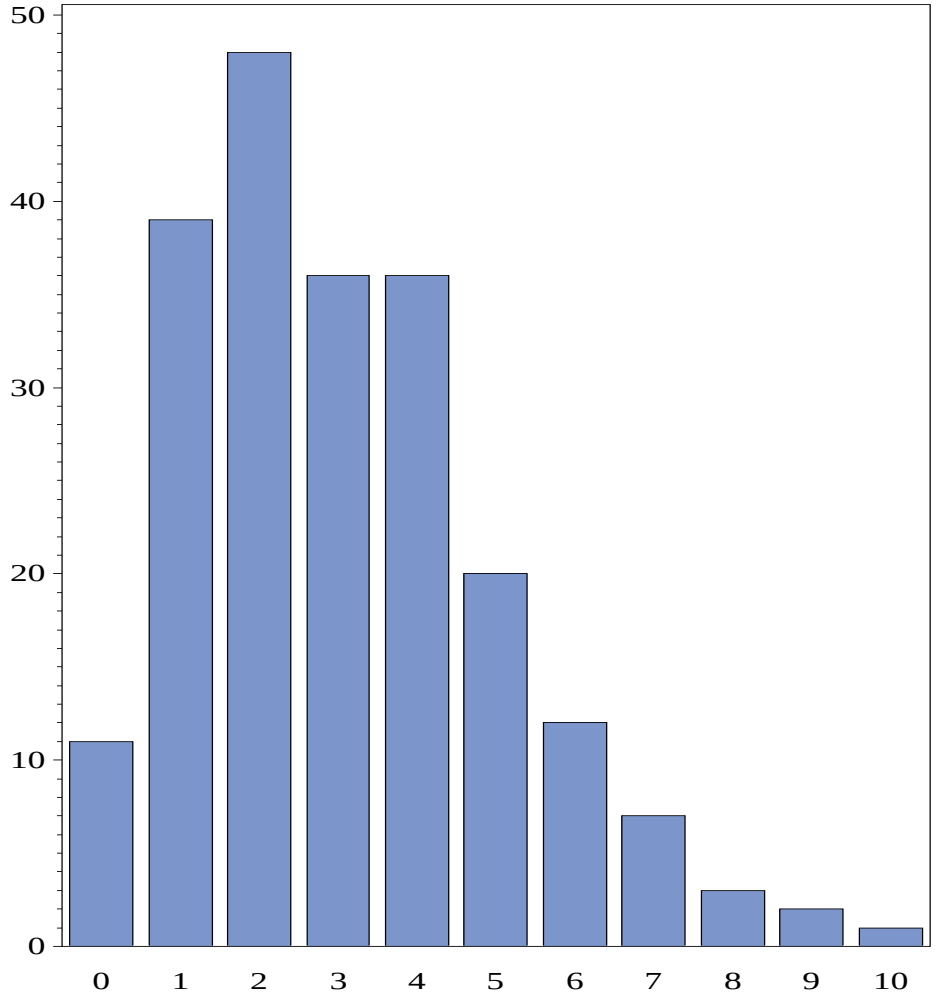
Ghosh *et al. Genome Biology* (2017) 18:225

Assessment of algorithm performance across different disease mechanisms



Ghosh *et al. Genome Biology* (2017) 18:225

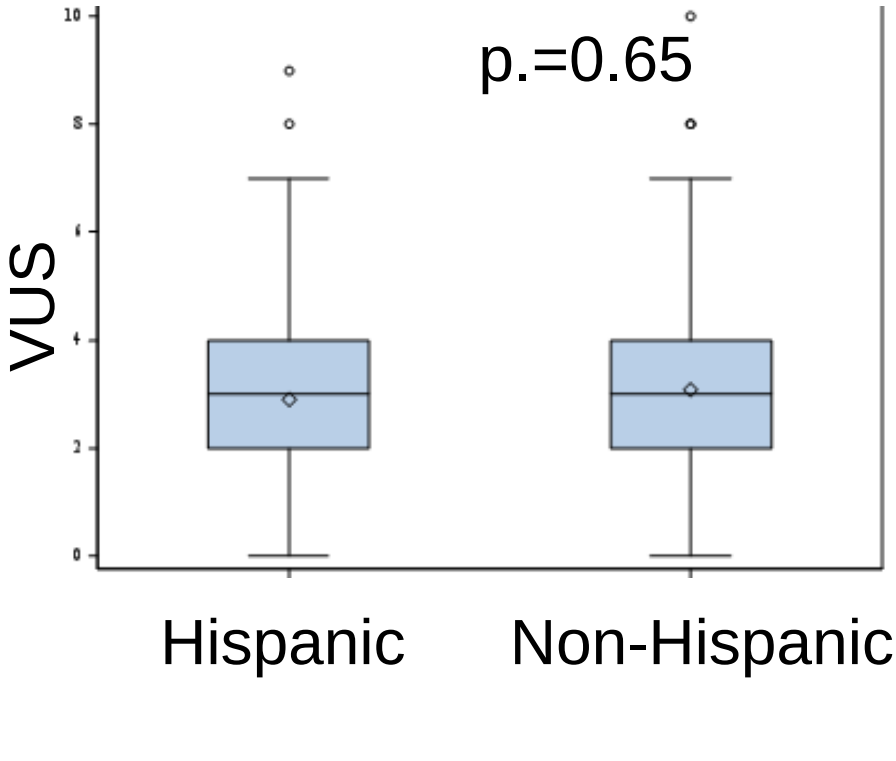
VUS REPORTED in CANCER SUSCEPTIBILTY GENES (n = 215 germline exome reports)

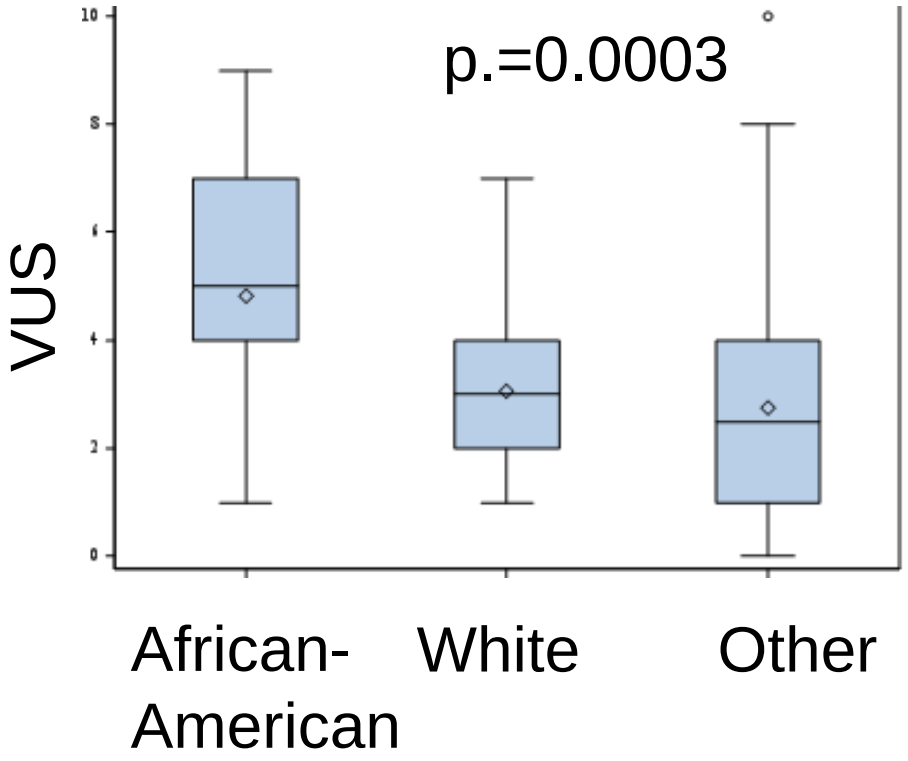


median of 3 VUS (range from 0 to 10)

Evaluation of VUS reports in cancer susceptibility genes based on:

- Ethnicity (Hispanic vs non-Hispanic) – median = 3
- Race - increased VUS reported in African-Americans – median = 5



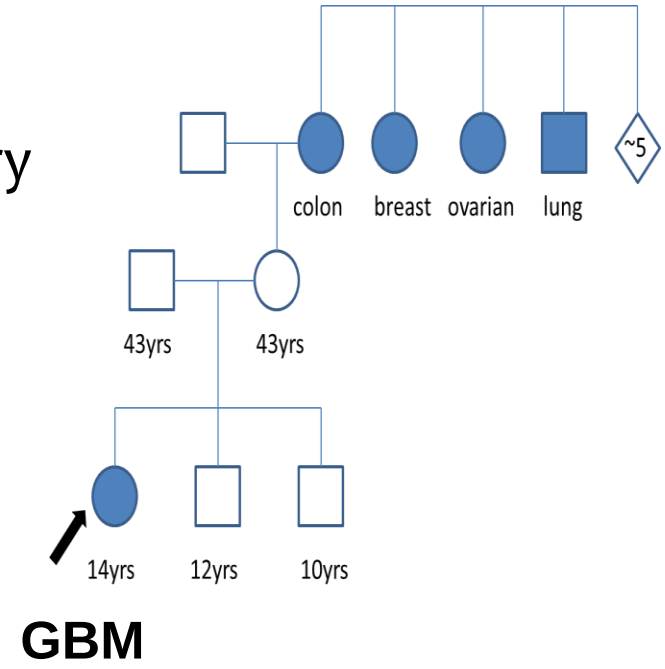


Cancer susceptibility molecular diagnosis in 9.8% (27/278) pediatric cancer patients

Autosomal dominant (P/LP)	**26**	**19 different genes**
Genes associated w/ specific childhood cancer	15	*Examples include DICER1, VHLx3, MSH2, WT1x2, TP53x3*
Genes not previously associated w/ specific childhood cancer	11	*Examples include BRCA1x2, BRCA2, PALB2, CHEK2x2, FLCN, SMARCA4*
Autosomal recessive (biallelic)	**1**	*TJP2*
No one gene was reported in more than 3 BASIC3 patients: 3 each for *VHL* and *TP53*.		

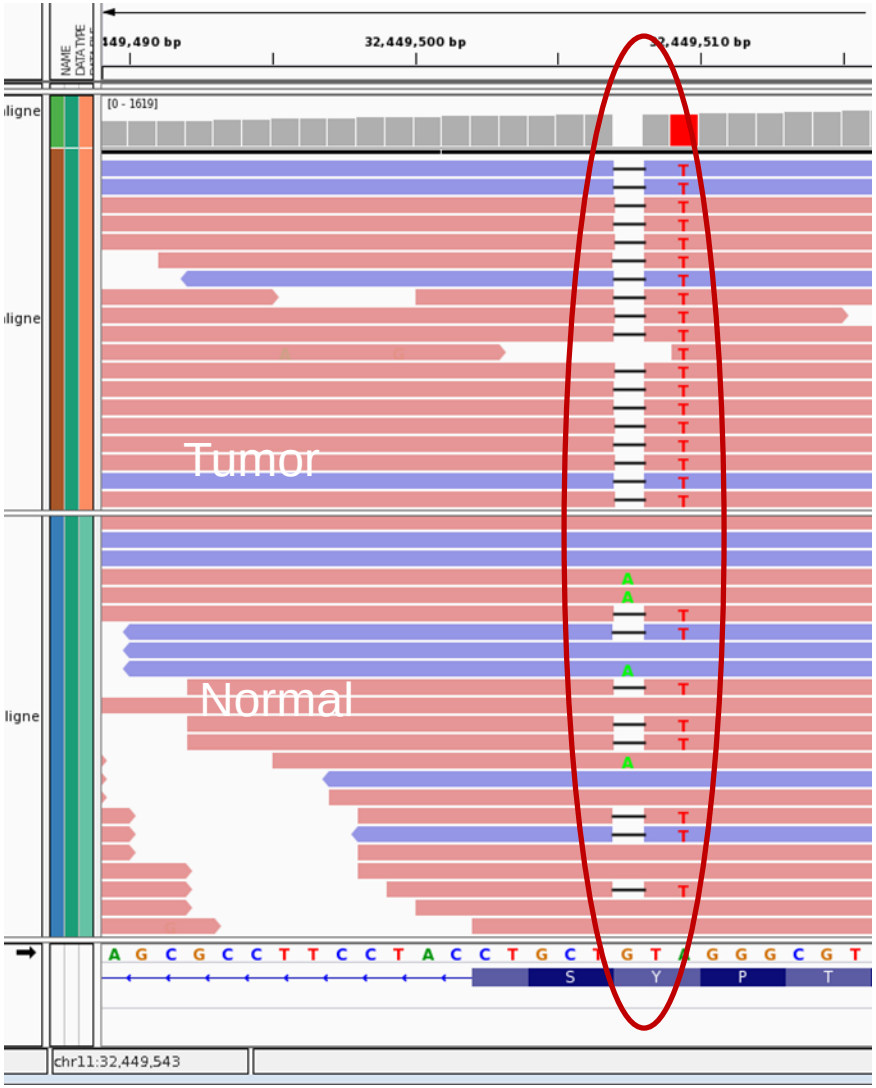
Germline results can have an impact on multiple family members

- 14 yo girl with glioblastoma
  - Mother aware of cancer family history but not in electronic medical record
  - Sequencing revealed **c.1697delA frameshift mutation in *MSH2*** transmitted from her mother.
- *MSH2* mutation associated with Lynch syndrome and glioma.
  - **Cancer screening recommendations** made for siblings, mother and other *MSH2* positive family members
  - Now important for **treatment** decisions



Example of unexpected finding of mosaic *WT1* mutation in patient with Wilms tumor

- **Subject 223202** – 9 mo male with Stage III Wilms tumor.
- No FH of cancer, no congenital anomalies and no genetic testing recommended.
  - WES revealed **mosaicism for frameshift in *WT1***.
  - Complete loss of heterozygosity in tumor.
  - Finding of *WT1* mutation resulted in long-term renal function assessment and more frequent contralateral kidney surveillance.



Angshumoy Roy

Newly described TSG with unexpected tumor: *SMARCA4* LOF w/ neuroblastoma tumor

Can we predict which patients have findings?

Column1	Cancer Diagnostic Finding	n=278	Yes (n=27) 9.8%	No (n=251)	p*
Age					0.6324
	<2	43	6 (14%)	37 (86%)	for trend
	2-12	159	14 (8.8%)	145 (91.2%)	
	>12	76	7 (9.2%)	69 (90.8%)	
Gender					0.6898
	Female	135	12 (8.9%)	123 (91.1%)	
	Male	143	15 (10.5%)	128 (89.5%)	
Ethnicity					0.8372
	Hispanic or Latino	133	12 (9%)	121 (91%)	
	Non-Hispanic	136	14 (10.3%)	122 (89.7%)	
	NA	9	1	8	
Race					0.6453
	White	159	13 (8.2%)	146 (91.8%)	
	Black	27	2 (7.4%)	25 (92.6%)	
	other (American Indian, Asian, >1 race)	37	1 (2.7%)	36 (97.3%)	
	NA	55	11	44	
Tumor type					1
	CNS	97	9 (9.3%)	88 (90.7%)	
	Non-CNS	181	18 (9.9%)	163 (90.1%)	

* p-values were calculated by Fisher's exact test

Also little correlation with histologic diagnosis except rare tumors, e.g. PHEO, PPB

	Cancer Diagnostic Finding			
HISTOLOGY	**n=278**	**Yes (n=27)**	**No (n=251)**	**p***
ATRT	4	1 (25%)	3 (75%)	0.337
CARCINOMA OTHER	14	3 (21.4%)	11 (78.6%)	0.144
CNS OTHER	20	1 (5%)	19 (95%)	0.704
EPENDYMOMA	11	0	11 (100%)	0.6081
EWING SARCOMA	13	1 (7.7%)	12 (92.3%)	1
GERM CELL TUMOR	24	0	24 (100%)	0.1449
HIGH GRADE GLIOMA	7	1 (14.3%)	6 (85.7%)	0.5149
LIVER TUMOR	9	2 (22.2%)	7 (77.8%)	0.2138
LOW GRADE GLIOMA	31	3 (9.7%)	28 (90.3%)	1
MEDULLOBLASTOMA	18	1 (5.6%)	17 (94.4%)	1
NEUROBLASTOMA	30	3 (10%)	27 (90%)	1
NON-CNS OTHER	23	7 (30.4%)	16 (69.6%)	0.0031
OSTEOSARCOMA	14	0	14 (100%)	0.3746
RHABDOMYOSARCOMA	15	1 (6.7%)	14 (93.3%)	1
SARCOMA OTHER	19	0	19 (100%)	0.2326
WILMS TUMOR	26	3 (11.5%)	23 (88.5%)	0.7271
any SARCOMA	61	2 (3.3%)	59 (96.7%)	0.083
SARCOMA w/out EWING	48	1 (2.1%)	47 (97.9%)	0.0587

Inheritance pattern of diagnostic mutations

Diagnostic finding	N=27
Parental Samples Available	20
Inherited from a parent	16
De novo (3) or mosaic (1)	4
Proportion inherited from a parent	**80%**

- 80% of alleles inherited!
- Equivalent maternal and paternal inheritance
- Parents have been very interested in having at-risk siblings tested for the mutations identified

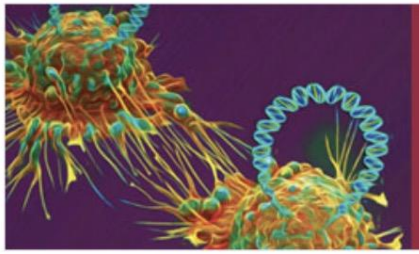
Early Data on Clinical Utility: Cancer Surveillance Recommendations for Germline Findings

Kindreds Impacted	Number
Patient and sibling	5
Parent only	7
Both	11
None	3

Examples of relevance

- Both parents & siblings:*TP53*, *VHL*
- Parents only: *BRCA1*, *CHEK2*
- No recommendations: *KRAS*, *PTPN11*, *TJP2*

- Cancer screening in siblings has been initiated through dedicated pediatric cancer screening clinic.
- Major focus of our CSER2 project.



CLINICAL CANCER RESEARCH

Pediatric Oncology Series

AACR American Association for Cancer Research

Clinical Cancer Research is proud to present a special collection of articles from the AACR Childhood Cancer Predisposition Workshop. The initial series of manuscripts was generated by an international cohort of leading pediatric cancer experts in order to provide recommendations for screening surveillance of childhood cancer predisposition syndromes in an effort to facilitate early detection and treatment of pediatric cancers. We hope you enjoy this series of freely available articles and continue to check back for additional relevant content and updated recommendations.

- **The future of surveillance in the context of cancer predisposition: through the murky looking glass.**
  Malkin D…Brodeur GM. *Clinical Cancer Research* November 2017.
- **Pediatric cancer predisposition and surveillance: an overview, and a tribute to Alfred G. Knudson Jr.**
  Brodeur GM…Malkin D. *Clinical Cancer Research* June 2017.
- **Pediatric cancer predisposition imaging: focus on whole-body MRI.**
  Greer MLC…States LJ. *Clinical Cancer Research* June 2017.
- **Recommendations for surveillance for children with leukemia-predisposing conditions.**
  Porter CC…Nichols KE. *Clinical Cancer Research* June 2017.
- **Recommendations for childhood cancer screening and surveillance in DNA repair disorders**
  Walsh MF…Savage SA. *Clinical Cancer Research* June 2017.
- **Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood.**
  Tabori U…Brugières L. *Clinical Cancer Research* June 2017.

Single pathogenic variants in genes for autosomal recessive cancer syndromes

- Total of 18/278 BASIC3 (6.5%) pediatric cancer patients had P/LP variants in a variety of recessive cancer syndrome gene.
- We subsequently reviewed medical findings at entry into study.
  - 0 of 18 subjects had clinical features of the recessive disorder except one patient with PFO and *FANCL* variant.
- Several of these reported variants were within Fanconi anemia genes (FANCC, FANCL, FANCM).

What is the expected frequency of Fanconi anemia pathway variants in pediatric patients undergoing WES?

- Evaluated the frequency of pathogenic or likely pathogenic (P/LP) variants in genes in the Fanconi pathway from Baylor clinical whole exome sequencing patients referred for non-cancer findings.

- We evaluated this frequency in each of 15 FA genes: *FANCA, B, C, D1/BRCA2, D2, E, F, G, I, J/BRIP1, L, N/PALB2, O/RAD51C, P/SLX4* and *BRCA1* (FA-like condition, *FANCS*)

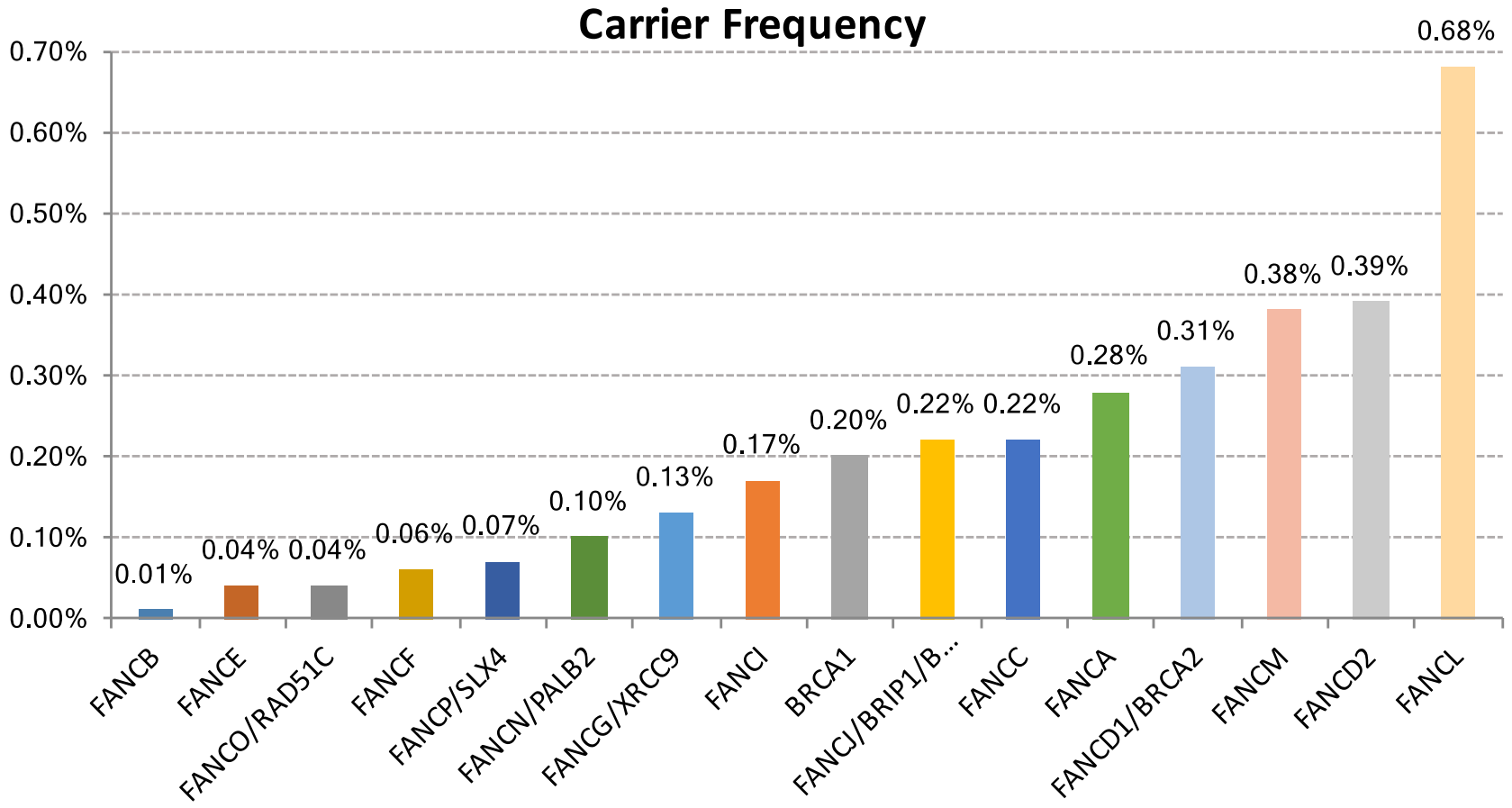
Clinical BCM non-cancer WES Cohort (n= 9986)

- As previously reported (Yang et al., *JAMA*, 2014) patients referred for clinical WES are predominantly in pediatric age range: 88% <18 years
- Referred for WES from a wide variety of medical centers.
- Most common indications are neurologic, intellectual disability and/or congenital anomalies.
- Data provided here is variants detected in proband:

Frequency of 3 autosomal dominant cancer susceptibility genes: *BRCA1, BRCA2, PALB2*

Gene	Heterozygous Patients	Carrier frequency
BRCA1	20	0.20%
BRCA2	31	0.31%
PALB2	10	0.10%

FA Carrier Status per Gene – summing across all FA gene = 2.92%



Nature of the pathogenic FA alleles found in non- cancer WES cohort

- 10% of *BRCA1* and 5% of *BRCA2* reported P/LP variants were missense alleles, whereas all other variants in FA genes were predicted to be truncating.

- Similarly, 90% of *BRCA1* and 92% of *BRCA2* mutations were previously reported in the literature where only 47% of the pathogenic variants in the other FA genes were previously reported.

Conclusions of Fanconi/BRCA Analysis

- Clinical WES of a large primarily pediatric cohort:
  - Approximately 2.9% are carriers of a Fanconi allele
  - This includes ~0.5% with either BRCA1 or BRCA2
- Now doing a comparison with Geisinger ~10K pediatric exomes to generalize the findings.
- This data provides framework for comparing findings in these genes in pediatric cancer cohorts, BASIC3, PCGP, TARGET, etc.

Clinical Expectations/Utility in BASIC3

- We prospectively evaluated whether standard clinical practice for genetic testing could predict the WES findings (or did the exome provide more information):
  - At entry, the BASIC3 clinical genetics team reviewed tumor pathology, family and medical history in the EMR and any study related surveys:
  - We determined if genetic testing would be considered for the patient based on clinical features?
  - If so, what genes or tests would be ordered?

Any testing considered?	#pts	Gene test considered	#pts
Yes	113	*TP53*	35
No	176	microarray	19

Katie Bergstrom, CGC Sarah Scollon, CGC and Sharon Plon, FACMG

We found poor ability to predict which BASIC3 subjects would have molecular diagnosis

- **Only 11 of 27 (41%) patients with diagnostic cancer susceptibility findings were predicted at entry.**
- **Variety of reasons subjects were missed:**
  - Didn't recommend testing for genes like *BRCA1*
  - Diagnoses that we might think are obvious (*PTPN11*/Noonan) were not considered by oncologists prior to the WES results.
  - Clinically, relevant molecular findings like *de novo* or *mosaic WT1* mutations in unilateral Wilms patients.

Need to anticipate ongoing evolution of variant interpretation (first reports in 2012)

- Child with pleomorphic xanthoastrocytoma and delayed speech
- History of tumors in maternal & paternal lineage
- Germline WES – pathogenic variant in *DKC1* - gene associated with dyskeratosis congenita
  - C.-142c>G in DKC1 shared by mother; reported in article in *Human Genetics* 2001 in patient with DKC and functional study showed that it disrupted sp1 binding site
- Referred to Alison Bertuch, who tested patient for peripheral blood telomere length, which was normal
- Now in gnomad database of 100K individuals
  - There are 16 hemizygotes (from ~50K males)
  - Unlikely this variant would be called pathogenic today

BASIC3 Conclusions and Recommendation

- Multiple studies demonstrate that ~10% of diverse pediatric cancer populations carry P/LP variants in wide range of dominant cancer susceptibility genes.
  - Mixture of genes with with and without prior association with the child's tumor diagnosis
  - Another ~6% carry single recessive alleles (no clear clinical significance or evidence of enrichment over controls).
- Current clinical practice for genetic evaluation may miss >50% of these children including clinically relevant germline findings for patient families.
- Time to develop clinical guidelines with germline panel/WES for all childhood cancer patients.

Contrasting WES results in pediatric cancer and neurodevelopmental cohorts

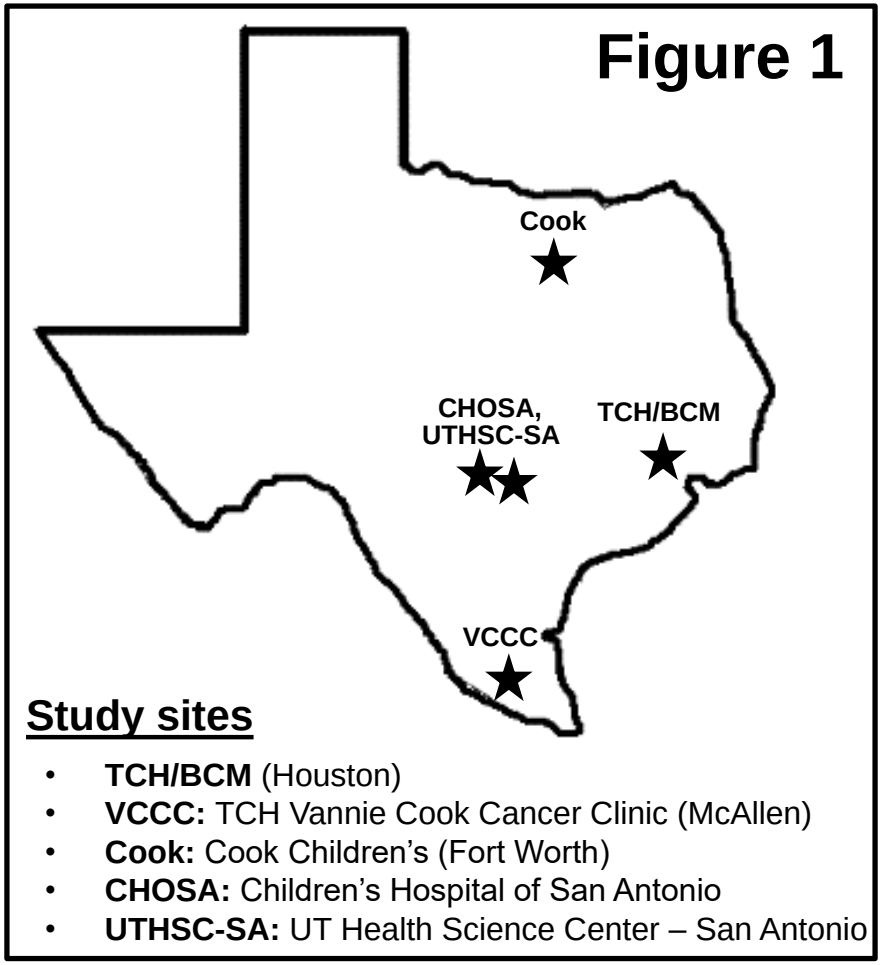
Pediatric Cancer

- Diagnostic rate of ~10%
- Autosomal dominant disorders predominate
- Small numbers but ~80% inherited from parent
- Results frequently impact screening & surveillance recommendations
- Tumor data can be used to aid interpretation of germline genome

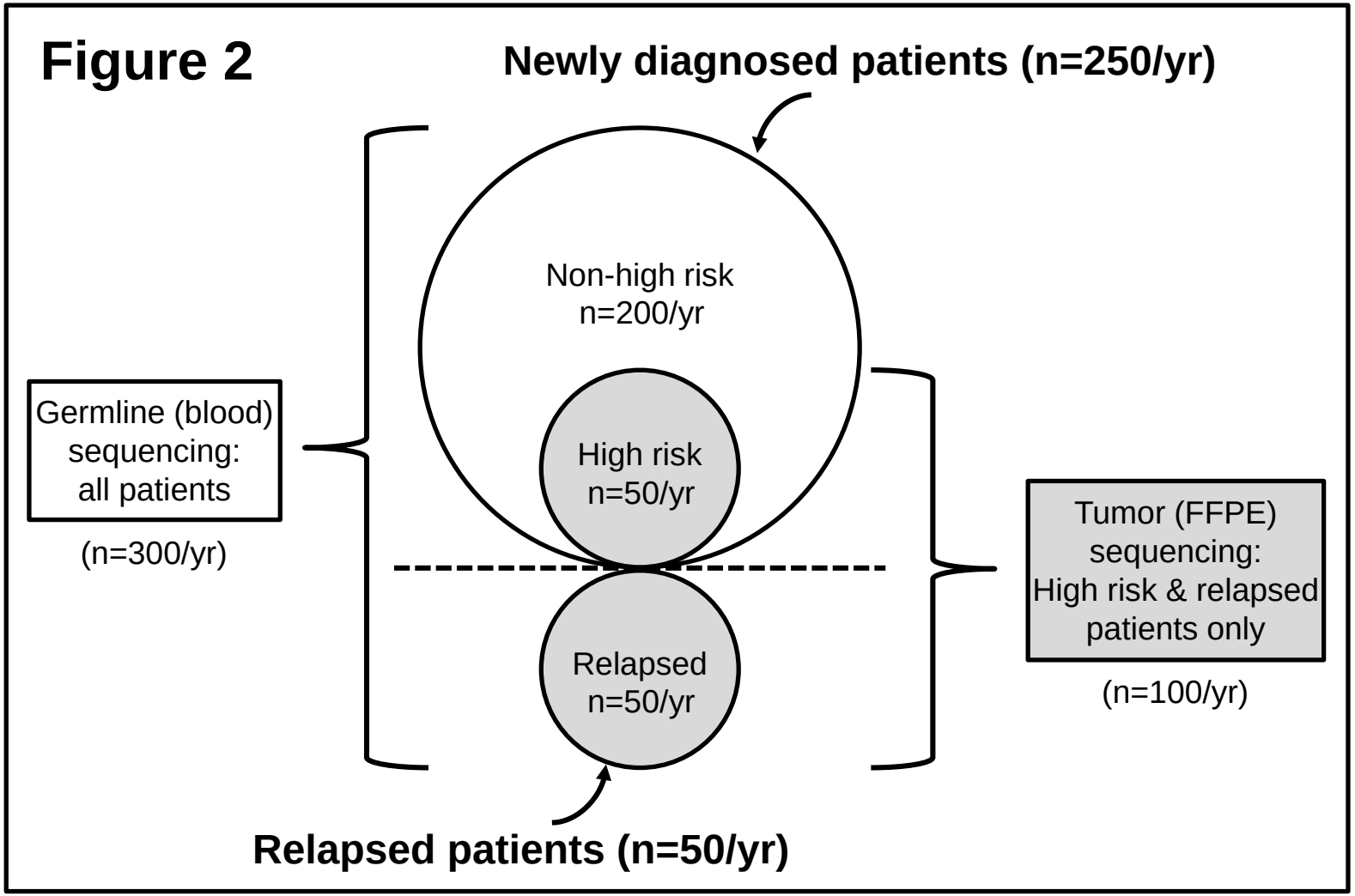
Neurodevelopmental

- Diagnostic rate of 25%
- More equal mixture of AD, AR and XLR
- *De novo* mutations (~70%) predominate (multiple DNM)
- Results used for diagnosis and refining recurrence risk for parents
- Relatively rapid identification of new germline disease genes

KidsCanSeq – Next phase of CSER project



Sequencing plan – direct comparisons of clinical utility with targeted panels



A Clinical Sequencing Exploratory Research (CSER) project
Supported by NHGRI/NCI 1U01HG006485

Baylor College of Medicine

HGSC
HUMAN GENOME SEQUENCING CENTER

Texas Children's Cancer Center

BASIC³ Project 1 (clinical)
- Sharon Plon, MD, PhD (Project PI)
- Will Parsons, MD, PhD (Project PI)
- Murali Chintagumpala, MD (co-I)
- Stacey Berg, MD (co-I,)
- Susan Hilsenbeck, PhD (co-I)
- Tao Wang, PhD (co-I)

BASIC³ Clinical Project Team
- TXCCC pediatric oncologists
- Robin Kerstein, MT, CCRA
- Sarah Scollon, MS, CGC
- Katie Bergstrom, MS, CGC
- Stephanie Gutierrez (Data manager)
- Ryan Zabriskie (Laboratory manager)

TCH/BCM Pathology
- Angshumoy Roy, MD, PhD
- Dolores López-Terrada, MD, PhD
- Adekunle Adesina, MD, PhD

TCH Surgery and Neurosurgery

BCM/TCH leadership
- David Poplack, MD
- Susan Blaney, MD
- Arthur Beaudet, MD
- James Versalovic, MD, PhD
- Jed Nuchtern, MD

BASIC³ Project 2 (sequencing and reporting)
- Richard Gibbs, PhD (co-PI)
- Christine Eng, MD (co-PI)
- Yaping Yang, PhD (co-I)
- Angshumoy Roy, MD, PhD (co-I)
- David Wheeler, PhD (Co-I)
- Donna Muzny, MS

BASIC³ Project 3 (ELSI)
- Laurence McCullough, PhD (co-PI)
- Richard Street, Jr., PhD (co-PI)
- Amy McGuire, JD, PhD (co-I)
- Melody Slashinski, PhD (co-I)

BASIC³

BCM **A**dvancing **S**equencing
Into **C**hildhood **C**ancer **C**are

PEC-MATCH study

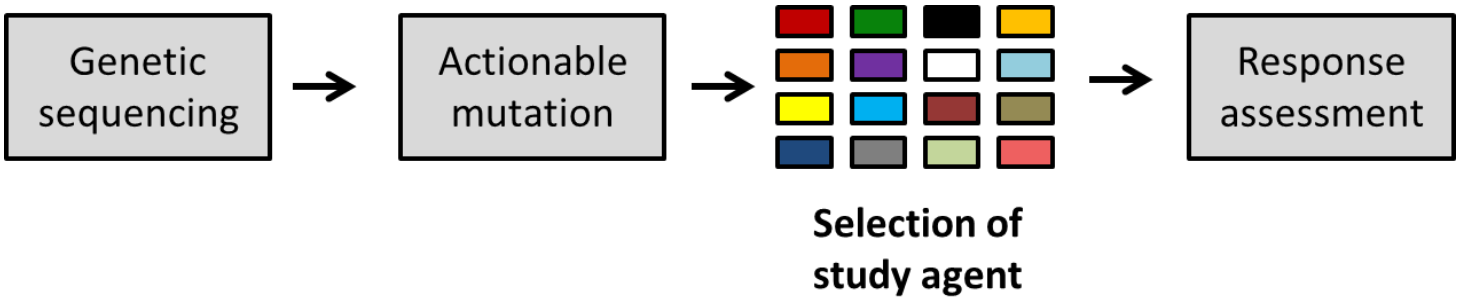
PROJECT:EVERYCHILD

CHILDREN'S ONCOLOGY GROUP

Precision oncology trial

NATIONAL CANCER INSTITUTE

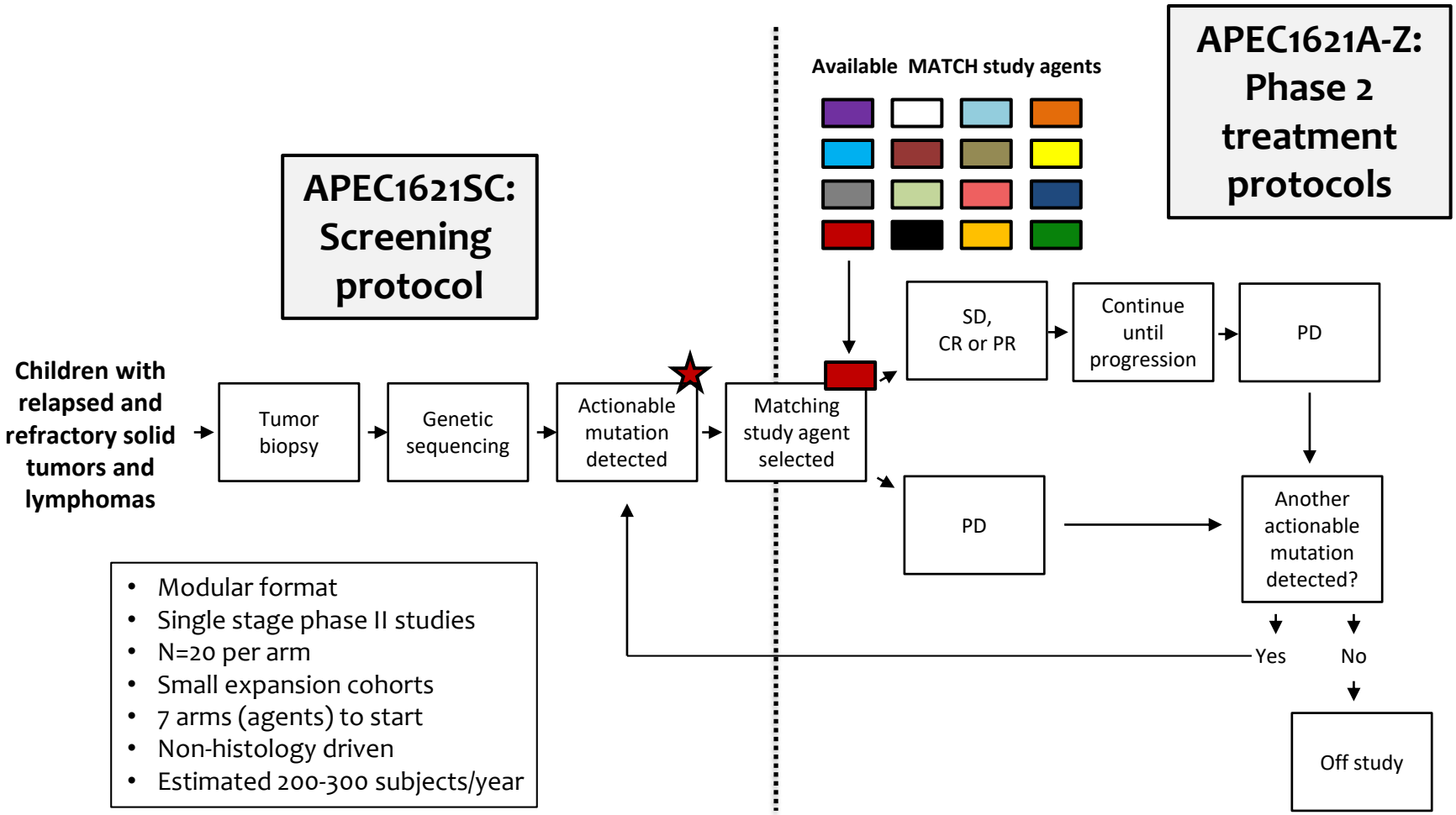
Objective: to open a COG-wide single stage phase II trial of genomically-directed therapies for children with refractory solid tumors and lymphomas



Primary objectives

- To determine the objective response rate in patients with *a priori* specified genomic alterations treated with pathway-targeting agents
- To determine the proportion of patients whose tumors have pathway alterations that can be targeted by existing drugs
- To demonstrate the feasibility of analyzing genetic pathway alterations in refractory/recurrent pediatric tumors in a timeframe that permits use of the results to guide therapy choices
- Germline analysis is not a primary objective

Study Overview



Clinical Sequencing

- FFPE tumor samples
- Oncomine DNA/RNA mutation panel (Life Technologies/ Thermo Fisher Scientific)
  - >140 genes
  - >4000 mutations of interest
  - defined set of SNVs, indels, CNVs, gene fusions
- Analytic pipeline adapted for pediatric study
- Sequencing to be performed at two existing NCI-MATCH laboratories
- Germline sequencing performed in parallel with results reported separately

Germline Reporting Committee Goals

- Mission: To devise and implement a procedure for the return of germline pathogenic cancer susceptibility mutation results (or other incidental findings) identified in study subjects.
- Specific tasks:
  - Develop a plan for the return of results obtained by clinical sequencing of study subjects
  - Develop a plan for the return of results (if indicated) from additional (non-clinical) research sequencing studies

Genes for germline reporting – those with known cancer susceptibility phenotype

gene	CNV	Hotspot	CDS	Fusion	Kinase domain	v.3 change (gene level)	Dominant	Recessive
ATM			YES				1	1
BRCA1			YES	YES			1	1
BRCA2			YES	YES			1	1
MLH1			YES				1	1
MSH2			YES				1	1
MSH6			YES			GENE ADDED	1	1
PALB2			YES			GENE ADDED	1	1
PMS2			YES			GENE ADDED	1	1
BAP1			YES				1	
CDKN2A	YES		YES	YES			1	
NF1			YES	YES			1	
NF2			YES				1	
POLE1			YES			GENE ADDED	1	
PTCH1			YES				1	
PTEN			YES	YES			1	
RAD51C			YES			GENE ADDED	1	
RAD51D			YES			GENE ADDED	1	
RB1			YES	YES			1	
SMARCA4			YES			GENE ADDED	1	
SMARCB1			YES				1	
STK11			YES				1	
TP53			YES	YES			1	
TSC1	YES		YES				1	
TSC2	YES		YES				1	
ALK	YES	YES		YES	YES		1	
CBL		YES					1	
CDK4	YES	YES					1	
CHEK2		YES					1	
EGFR	YES	YES		YES	YES		1	
GATA2		YES					1	
PTPN11		YES					1	
RET		YES		YES	YES		1	
SMAD4		YES					1	
TERT	YES	YES		YES			1	
FANCA			YES			GENE ADDED		1
FANCD2			YES			GENE ADDED		1
FANCI			YES			GENE ADDED		1
NBN			YES			GENE ADDED		1
RAD50			YES			GENE ADDED		1
SLX4			YES			GENE ADDED		1
ERCC2		YES				GENE ADDED		1
IDH1		YES						1
IDH2		YES						1

Summary of early MATCH germline results

- The steps needed to review and generate germline reports has been developed and put into place.
- Given recent studies we expect that most of the germline reports to be negative.
- Already have examples where germline reports (a) exclude possible diagnosis, (b) confirm known diagnosis or (c) provide unexpected cancer susceptibility information.
- Educational materials and website being developed.
- Genetics resource center to support oncologists receiving reports is available.

Project Updates - Enrollment

- 31 patients have been enrolled on the screening protocol (APEC1621SC) as of 10/31/17
- 18 patients have had tumor sequencing completed
- 5 patients have been matched to treatment protocols
- Already have examples where germline reports:

  (a) exclude possible diagnosis

  (b) confirm known diagnosis

  (c) provide unexpected cancer susceptibility information

NCI-COG Pediatric MATCH Study

Study committees

- Study design and logistics: Stacey Berg, Beth Fox
- Target/agent prioritization: Katie Janeway, Jae Cho
- Sequencing platform/analysis: Will Parsons, Jim Tricoli
- Germline result reporting: Sharon Plon, Steven Joffe
- Biospecimens: Julie Gastier-Foster
- Informatics: Hema Chaudhary, David Patton

COG leadership and staff

- Peter Adamson, Catalina Martinez, Rita Tawdros, Wendy Martinez, Todd Alonzo, Thalia Beeles, Heather Day…

NCI/CTEP leadership and staff

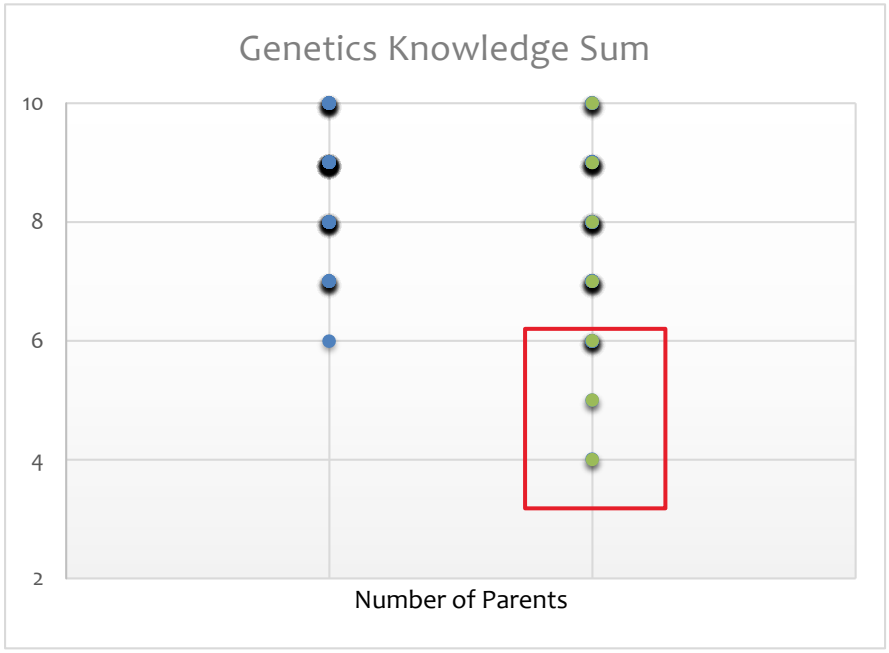
- Nita Seibel (NCI study PI), Malcolm Smith, adult NCI-MATCH leadership (Conley, Chen, Williams, Patton)….

FDA leadership

- Martha Donoghue, Greg Reaman

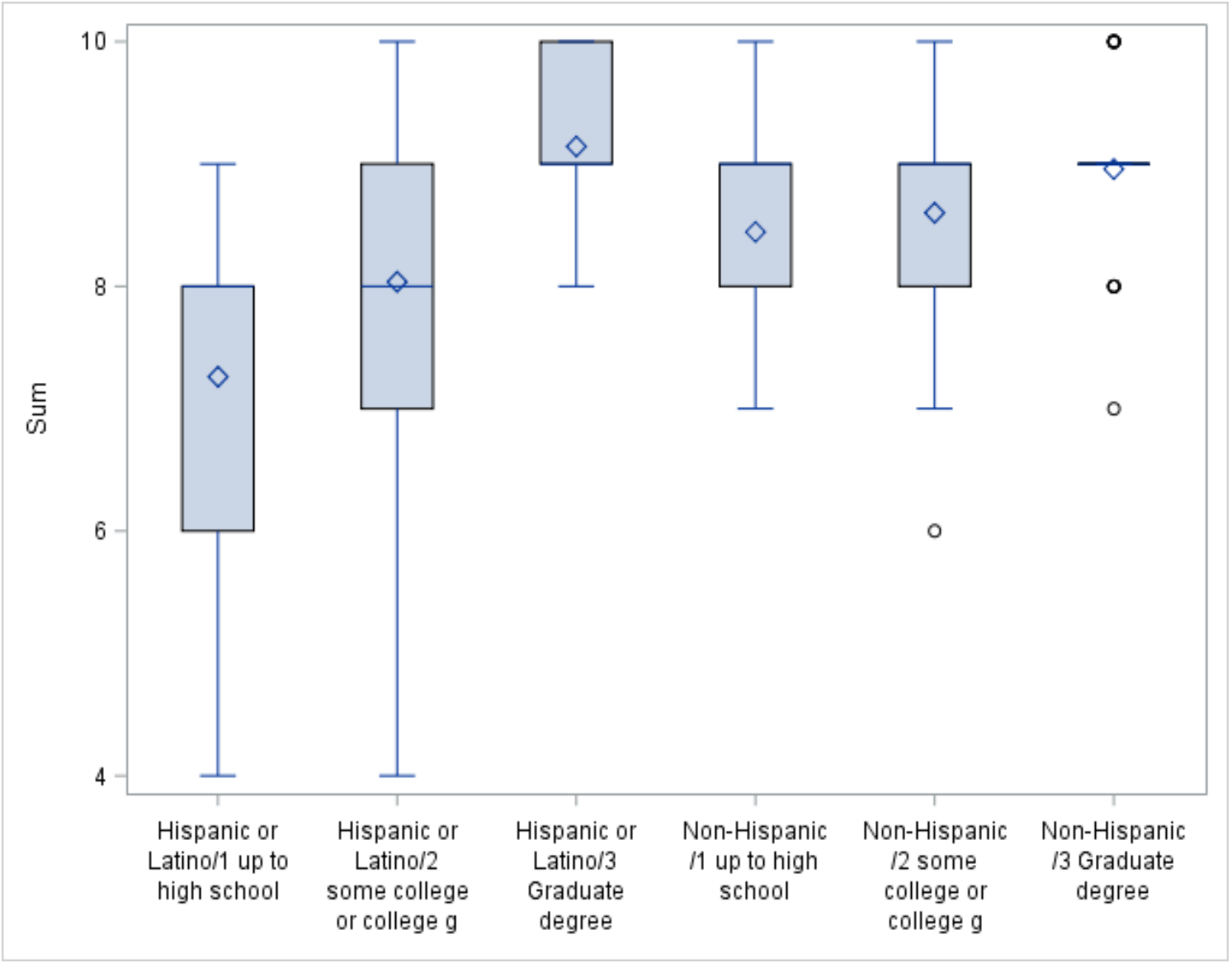
Questions?

Genetic knowledge and parental ethnicity



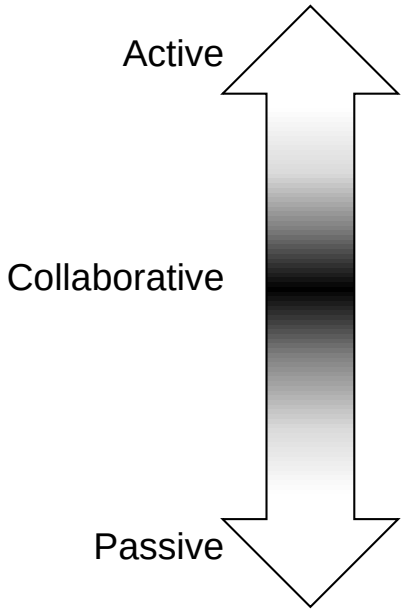
	All subjects		
Median	**Hispanic or Latino n=60**	**Non-Hispanic n=80**	**Wilcoxon rank sum test P**
Genetic knowledge	Range (4-10)	Range (6-10)	
Sum score	8	9	0.0002

Interaction between ethnicity, education, and genetic knowledge



Parents preferences for decision making role

Select the phrase that best describes the role you have actually taken with your child's doctor in dealing with your child's healthcare:



1. I prefer to make the final selection about which treatment my child will receive
2. I prefer to make the final selection of my child's treatment after seriously considering my child's doctor's opinion
3. **I prefer that my child's doctor and I share responsibility for deciding which treatment is best for my child**
4. I prefer that my child's doctor makes the final decision about which treatment will be used, but seriously considers my opinion
5. I prefer to leave all decisions regarding my child's treatment to my child's doctor