A Clinical Perspective on the Need for Integration

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Disclosures

- Past-President of the American College of Medical Genetics and Genomics (ACMG)
- PI on DOD funded grant on autism
- Chair of the external advisory board for the NIH funded Mouse Genome Informatics database, The Jackson Laboratory

Precision Medicine

- Possible through disruptive technology of NGS and advances in computational biology
- Clinical utility currently
  - Diagnosis of rare Mendelian disorders
  - Cancer diagnosis and personalized therapeutics
- Future expected clinical utility
  - Pharmacogenomics
  - Multifactorial disorders

Clinical Exome Sequencing

- High diagnostic yield (~25-40%)
- Importance of studying trios – higher yields in trios of ~40% vs ~25% if study DNA from proband only (peds)
- VUS and actionable secondary findings are common (the latter in ~1-5% of cases depending on lab)

Secondary Findings

Actionable secondary findings – damaging variants in disease genes unrelated to the reason testing was sent for which there is significant morbidity and/or mortality and where early dx can ameliorate or prevent the disease

Secondary Findings in Clinical Sequencing

- Recommendations of ACMG & President's Commission on Bioethics (2013) to search for and report them
- ACMG "Minimum list" of 56 **actionable** genes and specific mutations
  - Hereditary cancer genes, Marfan and related syndromes, inherited cardiomyopathies & arrhythmias, familial hypercholesterolemia, malignant hyperthermia
- Pathogenic variants in this gene list should be reported regardless of indication for clinical exome sequencing
  - Additional genes may be analyzed
  - Minimal list should be reported regardless of patient age
  - Patients/parents may "opt out" at time of consent

Secondary Findings

- Labs should seek and report only certain types of variants (pathogenic, likely pathogenic)
  - Low prior likelihood of disease for secondary findings
  - Labs should list quality of coverage/data which may be lower than for diagnostic genes
- Clinician/team has responsibility to provide appropriate pre- and post-test counseling [should include qualified genetics professional(s)]
- List should be refined and updated at least annually
- No consensus or recommendations on reporting of 2ary findings in research WES/WGS sequencing

Who are the Best Candidates for Clinical Exome Sequencing?

- Specific phenotypes/disorders should lead to specific genetic testing (single gene, gene panel)
  - May be less coverage of specific genes/regions on WES
  - Longer TAT; ?higher cost; lower % reimbursement
- Testing prior to exome (peds)
  - Microarray analysis - MCA, intellectual disability (IDD), severe szs, severe ASD (low IQ, dysmorphic); may uncover consanguinity
  - Low cost screening tests where appropriate

Utility of a Genetic Diagnosis

- Prevents additional unnecessary testing
- May help predict future medical complications
- May help tailor specific interventions
- May help predict function as an adult
- Will often provide better guidance concerning recurrence risks
- Will occasionally permit specific medical therapies that may significantly improve the outcome


NATIONWIDE CHILDREN'S

Models for Clinical Genomics - NCH

- All exome sequencing must be ordered or approved by a clinical geneticist
- Referrals to Genetics
  - Ongoing from multiple services, outside providers
- Case conference started with Neurology (9/14); GI (12/15); Endocrine (4/16)
- Genomics Clinic, planned 2016

Clinical Exome Sequencing Results at NCH from 10/29/12 – 8/3/15

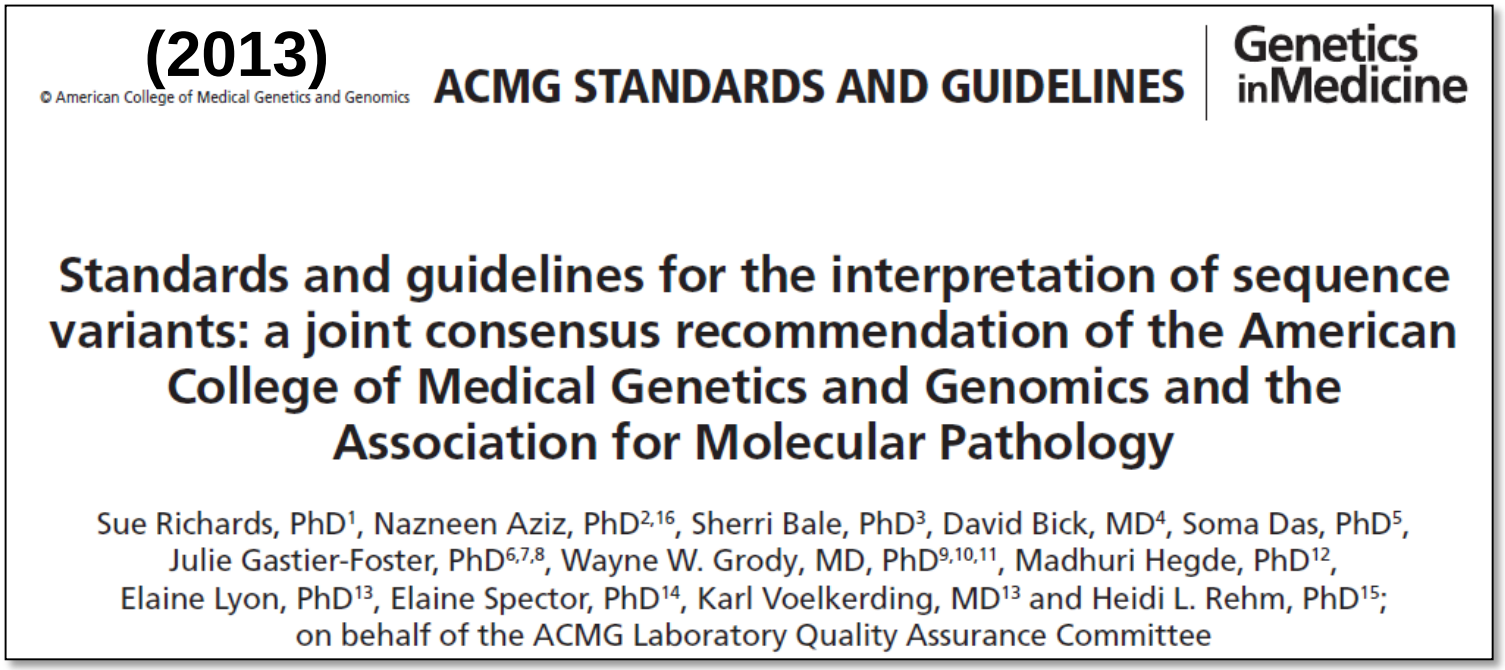
Exomes Completed (Baylor-Miraca)	160
Cause Identified (Pathogenic variant found related to disease)	71 (44%)
Likely Cause Identified (awaiting confirmation)	0
Questionable Results (VUS, pathogenicity unclear)	2
Actionable Secondary Findings (BRCA1, MEN I, BRCA2, KCNQ1)	4 (2.5%)

Implications for Management on 1st 100 Cases

- 19/41 (46%) with positive result had change in management beyond reproductive risk
  - 16/41 change in surveillance, including increased cancer risk (DKC)
  - 3/41 specific rx such as medication, diet (Lesch-Nyhan, AR disorder of creatine synthesis, novel sz/movement disorder)
- 20/41 clearly de novo – dramatic reduction in recurrence risk (?25% to <1%)
- 3 novel genes identified (PURA, VARS2, NR1H4 that encodes FXR)

Trends in Clinical Sequencing

- Expansion to carrier and population screening
- Move from gene identification to validation of variant pathogenicity; Need rapid, robust tools to validate potential disease-causing variants, particularly missense variants
- Move toward WGS, with assessment of chr rearrangements included in analysis; increased complexity of assessing non-coding variants



(2013)

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

- Standardized process for classifying variants
- Work group of Lab Directors and Clinicians from ACMG, AMP, CAP
- Classification Terminology – pathogenic, likely pathogenic, VUS, likely benign, and benign

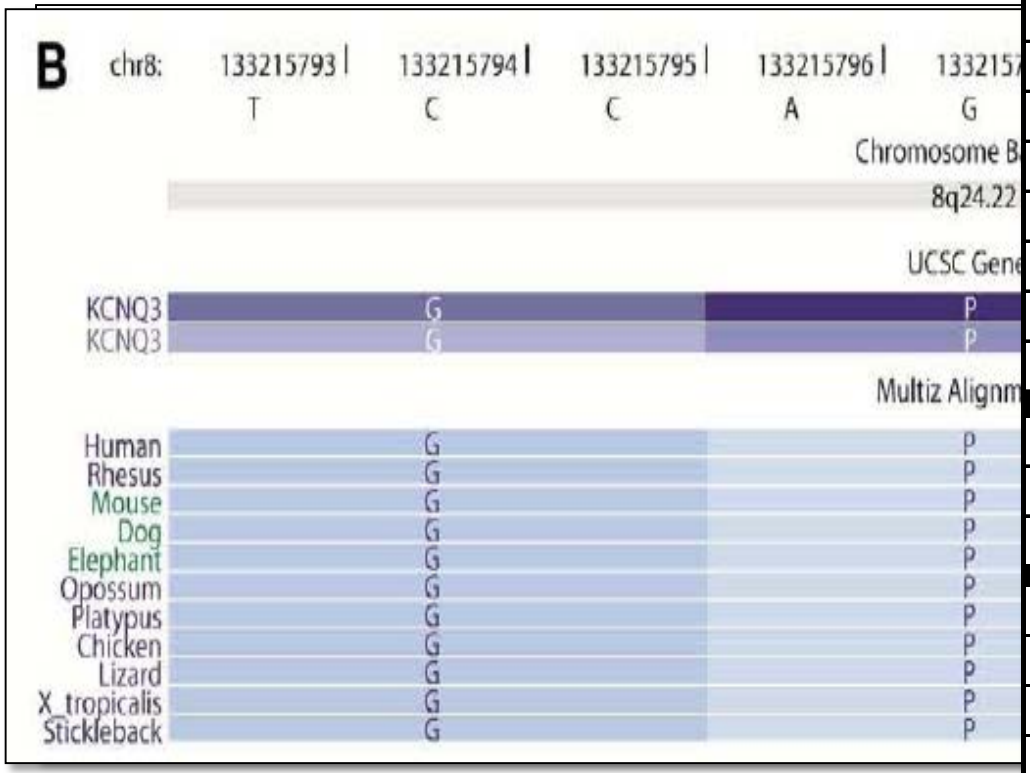
An Example

- 18 mo with progressive epilepsy; speech delay
- Seizure panel – no pathogenic variants; VUS *KCNQ3* c.1360C>T, p.Pro454Ser
- Gene causes AD seizure disorders – benign neonatal (BFNS), later onset szs
- 3 publications on this variant – suggestive functional data

- Eric Zmuda, Fellow, NCH Cytogenetics and Molecular Genetics Laboratory

Review of Evidence for *KCNQ3* c.1720C>T (p.Phe574Ser)

- Population frequency – Too high (?1:250 vs
- Case Control Study – Enriched in disease
- Conservation- Highly Conserved
- Functional Predictions – Conflicting



Feature	rs74582884
Location	8:132134369-132134369
Allele	A
Consequence	missense_variant
SYMBOL	KCNQ3
Gene	ENSG00000184156
Feature	ENST00000388996
BIOTYPE	protein_coding
EXON	13/15
CDS_position	1720
Protein_position	574
Amino_acids	P/S
SIFT	tolerated(0.05)
PolyPhen	probably_damaging(1)
GERP++	Conserved
phastCons7way_vertebrate	Conserved
phyloP7way_vertebrate	Conserved
Condel	deleterious(0.975)
MetaLR_pred	Deleterious
MetaSVM_pred	Deleterious
LRT_pred	Deleterious
PROVEAN_pred	Neutral
FATHMM_pred	Deleterious

Review of Evidence for *KCNQ3* c.1720C>T (p.Phe574Ser)

- ClinVar– Conflicting Interpretations
- Plug info into ACMG Checklist (modified online tool from ClinGen)….

Assertion and evidence details Go to:

ClinVar

Clinical assertions | Summary evidence | Supporting observations

Germline

Filter:

Clinical significance (Last evaluated)	Review status (Assertion method)	Collection method	Condition(s) (Mode of inheritance)	Origin	Citations	Submitter - Study name (Last submitted)	Submission accession
Benign (Jun 3, 2014)	criteria provided, single submitter (EGL Classification Definitions)	clinical testing	not specified [MedGen]	germline	PubMed (3) [See all records that cite these PMIDs]	Emory Genetics Laboratory (Jun 9, 2015)	SCV000113015.4
Uncertain significance (Jun 11, 2015)	criteria provided, single submitter (ACMG Guidelines, 2015)	clinical testing	not specified [MedGen]	germline		Genetic Services Laboratory, University of Chicago (Sep 15, 2015)	SCV000247669.1
Pathogenic (Apr 27, 2010)	no assertion criteria provided	literature only	Benign familial neonatal seizures 2 [MedGen | OMIM]	not provided	PubMed (1) [See all records that cite this PMID]	GeneReviews (Jan 8, 2013)	SCV000041085 .1

Review of Evidence for *KCNQ3* c.1720C>T (p.Phe574Ser)

ACMG Pathogenic Checklist June 2015 *Interactive Tool Developed by Lisa Susswein, lsusswein@genedx.com, May 2015; modified*

Suggested Classification: Pathogenic

Instructions: Only the highest strength category should be used for rules interpreting the same lines of evidence.

Use Checkboxes Below - Totals will Calculate Automatically. All numbers are "equal to or greater than."

No.	Criteria	Pathogenic	Likely Pathogenic
0	Very Strong	1 Very Strong AND	1 Very Strong AND 1 Moderate
2	Strong	1 Strong	1 Strong AND
0	Moderate	2 Moderate	1 Moderate
0	Supporting	1 Moderate and 1 Supporting	2 Supporting
		2 Supporting	3 Moderate
		2 Strong	2 Moderate AND 2 Supporting
		1 Strong AND	1 Moderate AND 4 Supporting
		3 Moderate	
		2 Moderate and 2 Supporting	
		1 Moderate and 4 Supporting	

Patient Name	
DNA #	
CoPath #	
Gene	KCNQ3
Variant	c.1720C>T (p.Phe574Ser)

Conclusion:

ACMG Guidelines “If the evidence for benign and pathogenic are conflicting, the variant defaults to uncertain significance.”

	Very strong	
☐	PVS1	Truncating variant (non
	Strong	
☐	PS1	Same amino acid chang
☐	PS2	De novo (both maternit
☑	PS3	Well established in vitr
☑	PS4	The prevalence of the v
	Moderate	
☐	PM1	Located in a mutational hot spot and/or critical and well-established functional domain.
☐	PM2	Absent from controls (or at extremely low frequency if recessive) in large samplesets (>1000 individuals) such as ESP & 1000 Genomes
☐	PM3	For recessive disorders, detected in trans with a pathogenic variant
☐	PM4	In frame deletions/insertions in a non-repeat region or stop-loss variant
☐	PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before [can also be used if pathogenic missense variant seen in same residue in highly analagous protein(s) (e.g. KRAS/NRAS/HRAS)]
☐	PM6	Presumed de novo (no confirmation of paternity and maternity)
	Supporting	
☐	PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease
☐	PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease
☐	PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
☐	PP4	Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology
☐	PP5	Reputable database reports variant as pathogenic but without evidence to independently evaluate. [acceptable databases defined for each gene but circular arguments should be avoided, e.g. lab's use of ClinVar when lab is the only submitter]

How Can Studies in Model Organisms Help?

- Demonstrate a role for protein in biological process
- (Help) demonstrate pathogenicity of a specific variant
- Examine gene-gene interactions
- Test potential therapies

Model of Choice Depends on Gene and Phenotype

- Yeast – conserved metabolic pathways
- Zebrafish – heart development, early nervous system development
- Xenopus – channel studies in oocytes
- Mouse – mammalian development (placenta, skeletal), learning & behavior
- Dog – certain tumors, behavior
- Primate – complex behaviors, language

Model of Choice Depends on Gene and Phenotype

- Yeast – conserved metabolic pathways
- X-linked mouse models of cholesterol synthesis disorders

Model of Choice Depends on Gene and Phenotype

- Mouse – mammalian development (placenta), behavior

- Damaging de novo variants in novel genes in 2 human autism pts - ?likely pathogenic based on behavioral phenotypes in KO mice

Using Mouse Model Data to Prioritize and Characterize Genes with Unknown Clinical Significance

Joanne Berghout, PhD
Outreach Coordinator
Mouse Genome Informatics

16 October 2015

- www.ACMG.net/EDUCATION
- Online Learning