



Department of Pathology

Perspectives on Existing Genetic Variation Resources From a Clinical Lab Director

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Levels of Analysis

Levels of Analysis

- Targeted exons
 - MEN2
- Single gene
 - CFTR, F8, Beta globin
- Gene panels
 - Cancer syndromes, cardiomyopathies, hearing loss, mitochondrial,
- Exome/genome level

Regions Interrogated

Exons

- Intron/exon boundaries
- Known intronic mutations
- Gene regulatory elements
 - 5' region, promoter
 - 3' UTR





ACMG Recommendations

• Report clinical significance

- "… the laboratory must provide the interpretive information and a best estimate of clinical significance for the variants…."
 - ACMG recommendations for standards and interpretation and reporting of sequence variations. Richards et al. Genet Med 2008 10:294-300





Mutation Classifications

Previously reported

- Pathogenic vs Benign
- Autosomal vs X-linked
- Recessive vs Dominant
- Previously unreported
 - Expected pathogenic
 - Suspected pathogenic
 - Uncertain
 - Suspected benign
- Further classification
 - Severe, moderate, mild, very mild

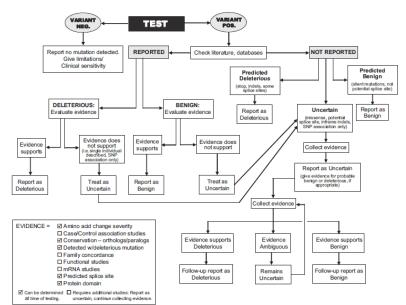


Fig. 1. A decision tree for interpretation of sequence variants and clinical reporting. Evidence that can be used to support sequence variant interpretation is shown in the box at the bottom.







Mutation Classification

- Check internal database
 - Differences between labs
- Locus-specific databases
 - Difference between databases, evidences given, updates, standards for classifications
 - Check original sources
- dbSNP, frequency (gene centric)
 - Benign and pathogenic mutations included
- Prediction algorithms (Polyphen-2, Sift, others)
 - no composite
- Literature search/ Google

PROBLEM: Don't know when to stop / what we've missed



Evidences

- Phenotype/Genotype
 - Cases/symptoms
 - Normal controls
- Functional studies

551000145	lound.						
Location	▲ Mutation Type ▼	Nucleotide Change	Protein Change	Transport Activ	vity Expression	References	Comments
5'UTR	Polymorphism	c207G>C				Peltekova et al. 2004	
5'UTR	Uncertain	c185A>C		33		Calderon et al., unpublished	
5'UTR	Uncertain	c149G>A		33		Calderon et al., unpublished	
5'UTR	Deletion	c91_22del				Nezu et al. 1999	
5'UTR	Polymorphism	c78C>T		33		Koizumi et al., 1999	
5'UTR	Polymorphism	c77G>A		33		Koizumi et al., 1999	
5'UTR	Polymorphism	c38A>C				Calderon et al., unpublished	
Exon 1	Missense	c.3G>T	p.M1I	<5		Dobrowolski et al. 2005	
Exon 1	Insertion	c.4_5insC	p.R2PfsX136			Nezu et al. 1999	
Exon 1	Nonsense	c.12C>G	p.Y4X	<1		Wang et al. 2001	
Exon 1-8	Deletion	c.33_1427del	p.G12_L477del	2 L	arge deletion fou	nd in two patients. Patient	
Exon 1	Missense	c.43G>T	p.G15W	3 1	of Italian desce	ent was heterozygous for this 5218VfsX68. Patient 2 of	
Exon 1	Missense	c.51C>G	p.F17L	14 🕨	Mexican descent was heterozygous for this		
Exon 1	Missense	c.56G>C	p.R19P	<5	mutation and p.T219SfsX20.		
Exon 1	Missense	c.59T>A	p.L20H			Calderon et al., unpublished	
Exon 1	Deletion	c.67_69deITTC	p.F23del	2		Lamhonwah et al. 2002	
Exon 1	Missense	c.83G>T	p.S28I	<12		Rahbeeni et al. 2002	

- Amino acid severity/splice predictors
- Conservation over species/gene families

95 records found

- Co-occurrence with causative mutations
 - Recessive vs dominant diseases
 - Chromosome phase
- Genetic evidence/Family concordance
 - Large family
 - Multiple small families





Collecting Evidences

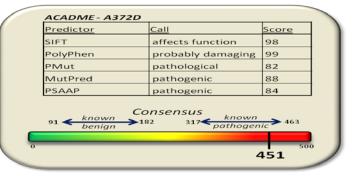
Testing additional family members

De novo

BORATORIES

- Linkage analysis
- Indirect measures (prediction programs)

PolyPhen benign 27 PMut neutral 7 MutPred benign 45	<u>Predictor</u>	Call	Score
PMut neutral 7 MutPred benign 45 PSAAP benign 4 Consensus known	SIFT	tolerated	21
MutPred benign 45 PSAAP benign 4 Consensus known	PolyPhen	benign	27
PSAAP benign 4 Consensus known	PMut	neutral	7
Consensus known	MutPred	benign	45
	PSAAP	benign	4





Learning

Courtesy of David Crockett, PhD

PREDICTED PATHOGENIC

Collecting Evidences

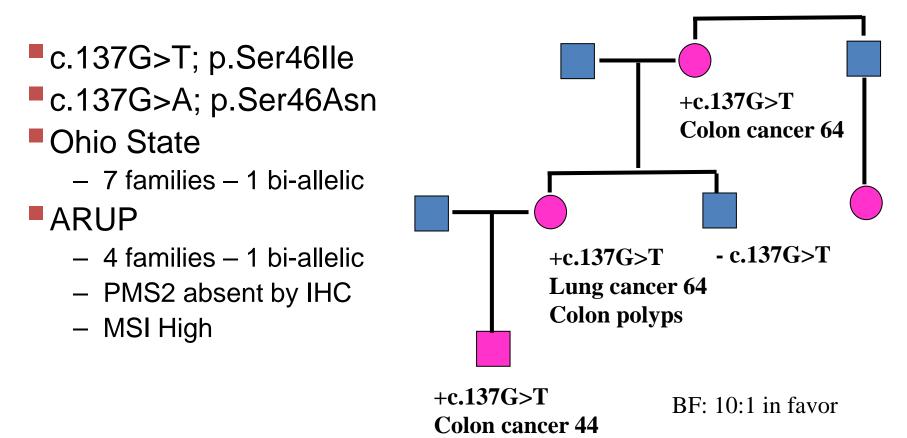
- Functional evidence
 - Histopathology
 - IHC
 - PMS2
 - Enzymatic/pathway analysis
 - MCAD: acylcarnitines
 - OCTN2: transport activity (fibroblasts); mutant expression
 - Structural analysis
 - RNA

ACMG recommendations. Genet Med 2008 10:294-300 2008





PMS2 Uncertain Variant



Pedigree from Leigha Senter-Jamieson, Ohio State University





Further Evidences

- AA predictions
- PolyPhen:Probably damaging (most severe class)
- Pmut: Benign, Reliability = 4 (of 10)
- PhD-SNP:Disease causing, Reliability Index = 8 (of 10)
- nsSNPAnalyzer: Disease causing
- AlignGVGD:class C65 mutation (most likely class to interfere with function)
- Conserved, but not strongly

Learning

- Not seen in 182 control chromosomes
- Western blot showed 50% protein compared to control
- Haploid-converted clones showed expression from only 1 allele



Nakagawa H et al. CANCER RESEARCH 64, 4721-4727, 2004



Laboratories Collecting

Patient Clinical History

- Symptoms
- Family history
- Previous lab results
- Molecular Results
 - Sequence variants
 - Common polymorphisms
 - Deletion/duplication analysis
- Re-classify variants
 - Variants of Uncertain Significance (VUS) to Benign, Pathogenic

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Ideal Clinically Valid Genome Database

Variants

- Pathogenic, Uncertain, Benign
- Severities, if known
- Ethnicities/Frequencies
- Number of cases (not necessarily multiple entries/variant)
- Symptoms
- In conjunction with other mutations
- Evidences
 - Not weighted equally
 - Risks of incorrect classification not equal between genes
 - Do not over-simplify
- Reasonable submission







Institute _{for} Learning



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