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UNIVERSITY OF UTAH
SCHOOL OF MEDICINE
Department of Pathology

Perspectives on Existing Genetic Variation Resources From a Clinical Lab Director

Elaine Lyon, Ph.D., FACMG
Associate Professor of Pathology
University of Utah
Medical Director, Genetics Division
ARUP Laboratories

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

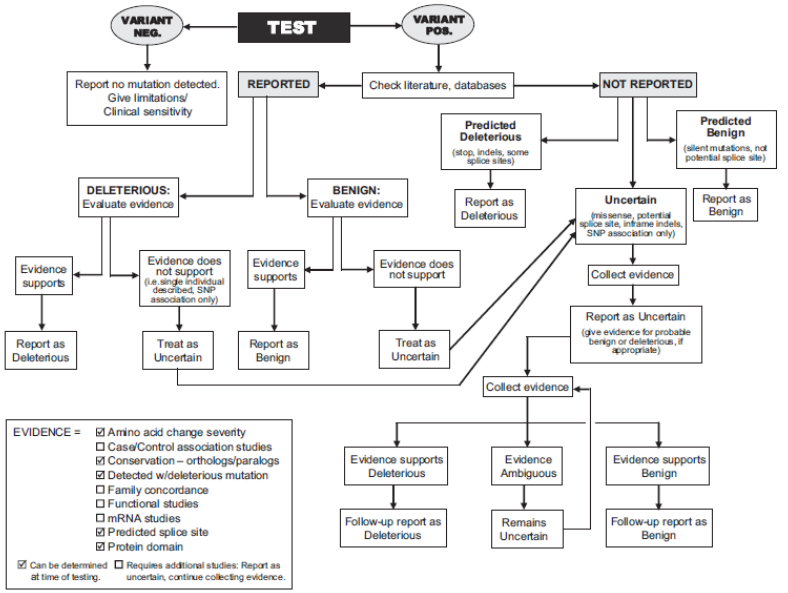


Fig. 1. A decision tree for interpretation of sequence variants and clinical reporting. Evidence that can be used to support sequence variant interpretation is shown in the box at the bottom.

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
- Amino acid severity/splice predictors
- Conservation over species/gene families
- Co-occurrence with causative mutations
  - Recessive vs dominant diseases
  - Chromosome phase
- Genetic evidence/Family concordance
  - Large family
  - Multiple small families

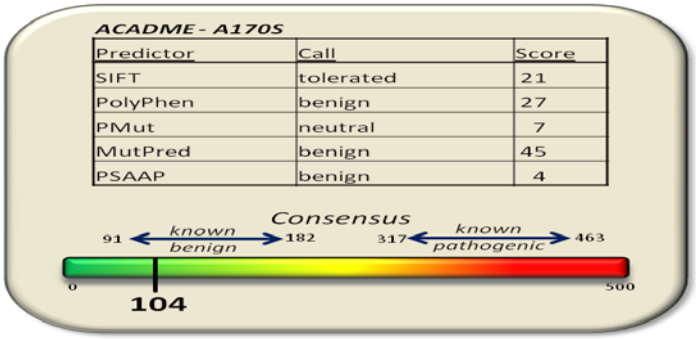
95 records found.

Location	Mutation Type	Nucleotide Change	Protein Change	Transport Activity	Expression	References	Comments
5'UTR	Polymorphism	c.-207G>C				Peltekova et al. 2004	
5'UTR	Uncertain	c.-185A>C		33		Calderon et al., unpublished	
5'UTR	Uncertain	c.-149G>A		33		Calderon et al., unpublished	
5'UTR	Deletion	c.-91_22del				Nezu et al. 1999	
5'UTR	Polymorphism	c.-78C>T		33		Koizumi et al., 1999	
5'UTR	Polymorphism	c.-77G>A		33		Koizumi et al., 1999	
5'UTR	Polymorphism	c.-38A>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			
Exon 1	Missense	c.43G>T	p.G15W	3			
Exon 1	Missense	c.51C>G	p.F17L	14			
Exon 1	Missense	c.56G>C	p.R19P	<5			
Exon 1	Missense	c.59T>A	p.L20H			Calderon et al., unpublished	
Exon 1	Deletion	c.67_69delTTC	p.F23del	2		Lamhonwah et al. 2002	
Exon 1	Missense	c.83G>T	p.S28I	<12		Rahbeeni et al. 2002	

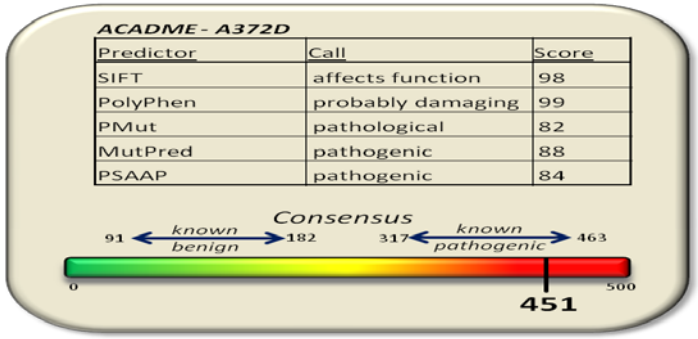
Large deletion found in two patients. Patient 1 of Italian descent was heterozygous for this mutation and p.G218VfsX68. Patient 2 of Mexican descent was heterozygous for this mutation and p.T219SfsX20.

Collecting Evidences

- Testing additional family members
  - De novo
  - Linkage analysis
- Indirect measures (prediction programs)



PREDICTED BENIGN



PREDICTED PATHOGENIC

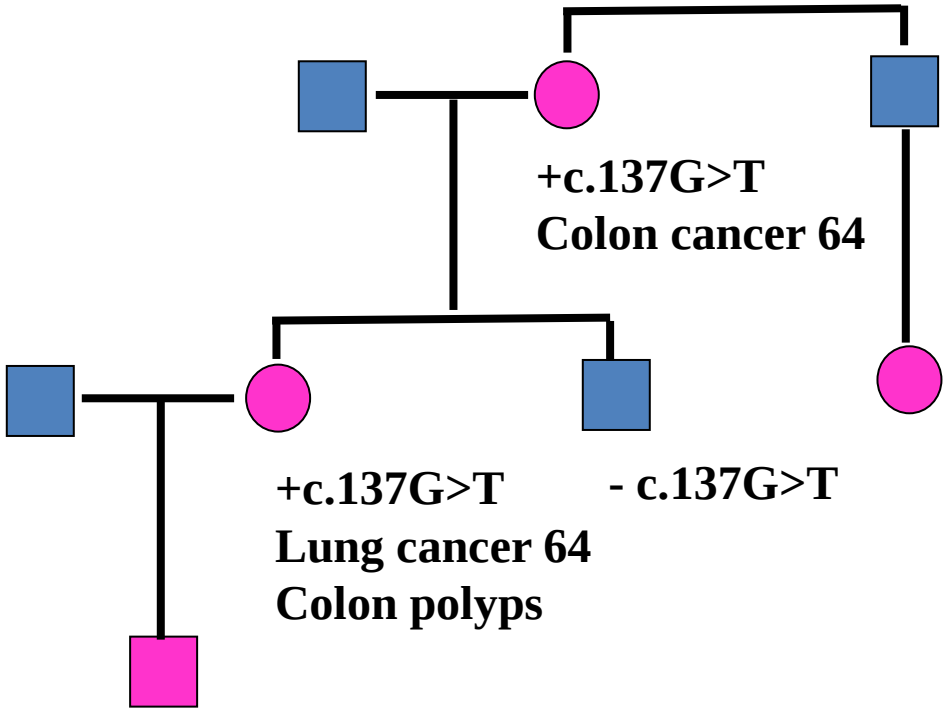
Collecting Evidences

- 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

- c.137G>T; p.Ser46Ile
- c.137G>A; p.Ser46Asn
- Ohio State
  - 7 families – 1 bi-allelic
- ARUP
  - 4 families – 1 bi-allelic
  - PMS2 absent by IHC
  - MSI High



BF: 10:1 in favor

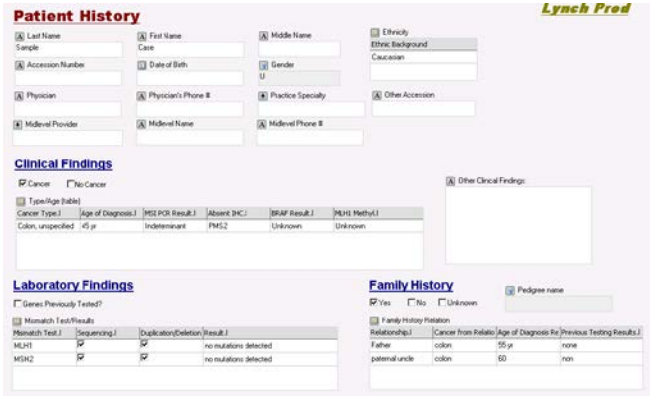
Pedigree from Leigha Senter-Jamieson, Ohio State University

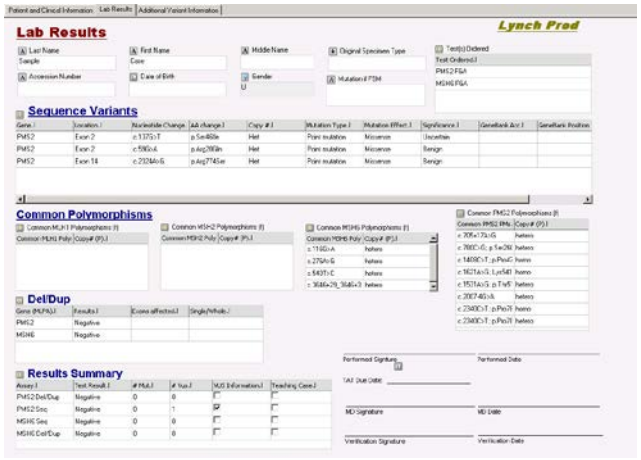
Further Evidences

- 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
- 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 Information





- 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

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

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