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UNIVERSITY OF UTAH
SCHOOL OF MEDICINE

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

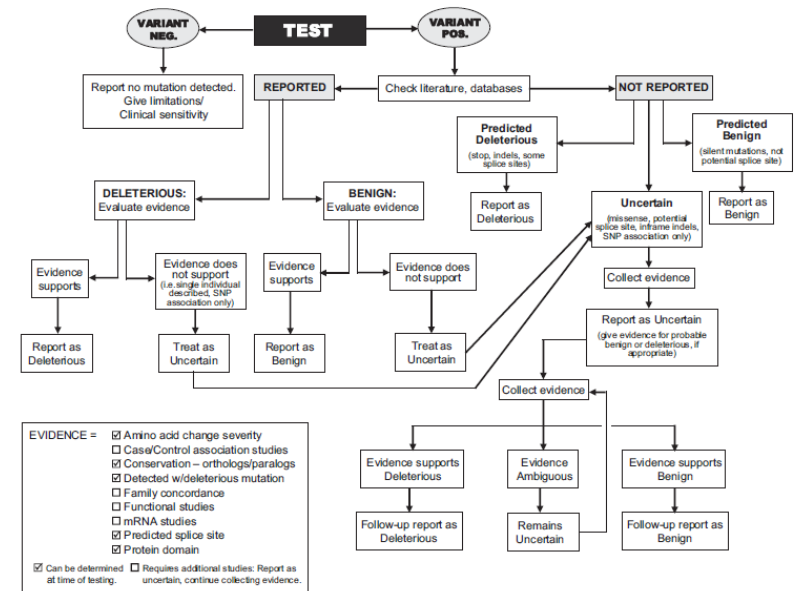


Fig. 1. A decision tree for interpretation of sequence variants and clinical reporting. Evidence that can be used to support sequence variant interpretation is shown in the box at the bottom.

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

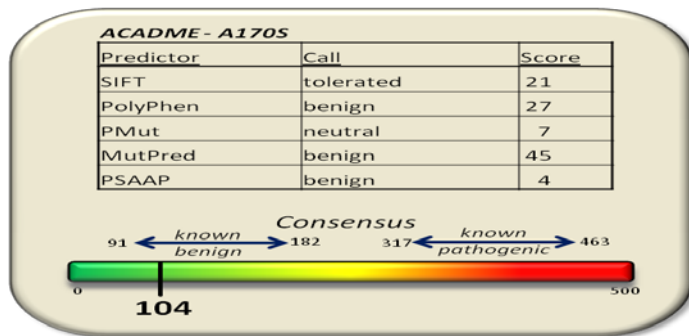
- Phenotype/Genotype
 - Cases/symptoms
 - Normal controls
- Functional studies
- Amino acid severity/splice predictors
- Conservation over species/gene families
- Co-occurrence with causative mutations
 - Recessive vs dominant diseases
 - Chromosome phase
- Genetic evidence/Family concordance
 - Large family
 - Multiple small families

95 records found.

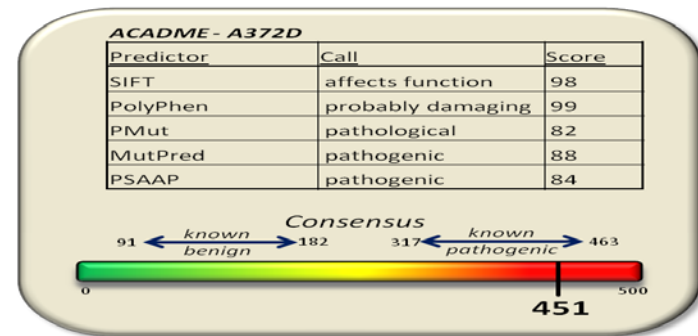
Location	Mutation Type	Nucleotide Change	Protein Change	Transport Activity	Expression	References	Comments
5'UTR	Polymorphism	c.-207G>C				Peltekova et al. 2004	
5'UTR	Uncertain	c.-185A>C		33		Calderon et al., unpublished	
5'UTR	Uncertain	c.-149G>A		33		Calderon et al., unpublished	
5'UTR	Deletion	c.-91_22del				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		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.T2195fsX20.	
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_69delITTC	p.F23del	2		Lamhonwah et al. 2002	
Exon 1	Missense	c.83G>T	p.S28I	<12		Rahbeeni et al. 2002	

Collecting Evidences

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



**PREDICTED
BENIGN**



**PREDICTED
PATHOGENIC**

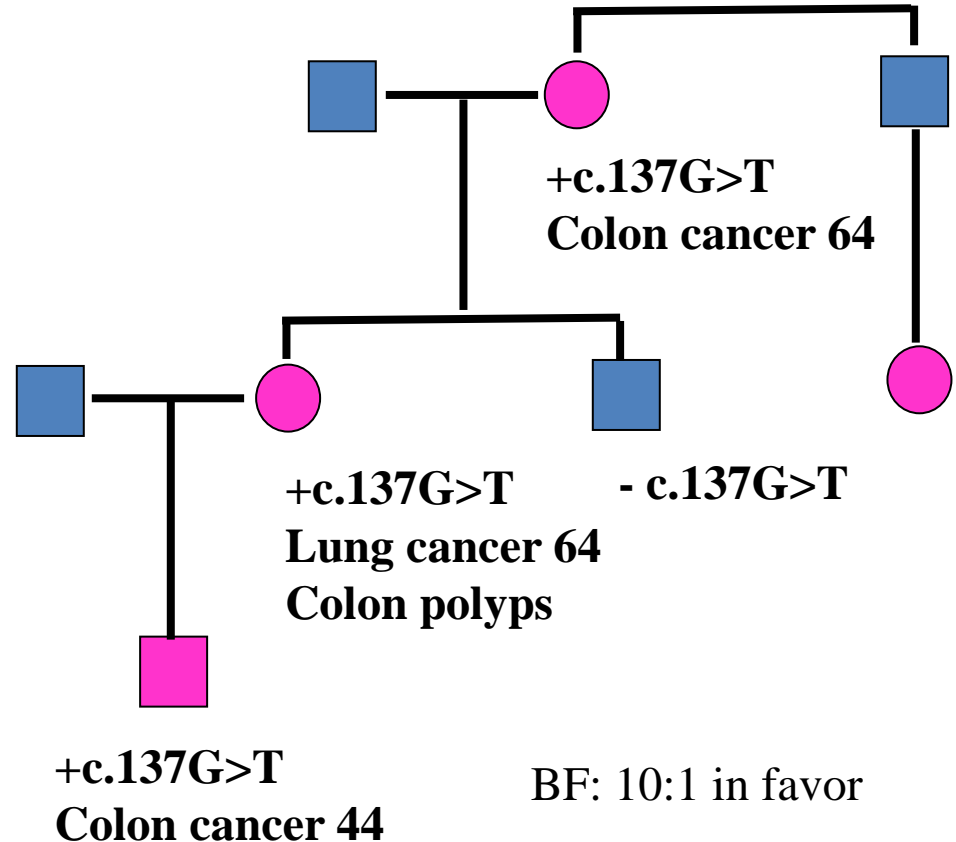
Collecting Evidences

- 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



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

Lynch Pred

Patient History

Last Name First Name Middle Name Ethnicity
 Sample Case Ethnic Background
 Accession Number Date of Birth Gender Caucasian
 Physician Physician's Phone # Practice Specialty Other Accession
 Midlevel Provider Midlevel Name Midlevel Phone #

Clinical Findings

Cancer No Cancer
 Type/Age (table) Other Clinical Findings:

Cancer Type	Age of Diagnosis	MSI PCR Result	Absent IHC	BRAP Result	MLH1 Methyl
Colon, unspecified	>50 yr	Indeterminate	MSI2	Unknown	Unknown

Laboratory Findings

Genes Previously Tested? Family History
 Match Family History Mutation
 Mismatch Test Results Cancer from Relative (Age of Diagnosis by Previous Testing Results)

Mismatch Test	Sequencing	Duplication/Deletion	Result	Relationship	Cancer from Relative	Age of Diagnosis	Previous Testing Results
MLH1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	no mutations detected	Father	colon	55 yr	none
MSH2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	no mutations detected	paternal uncle	colon	50	none

Family History

Pedigree name
 Family History Mutation
 Cancer from Relative (Age of Diagnosis by Previous Testing Results)

Lynch Pred

Lab Results

Last Name First Name Middle Name Original Specimen Type Test(s) Ordered
 Sample Case Test Order(s)
 Accession Number Date of Birth Gender Mutation PTSM MSH1/MSH2

Sequence Variants

Gene	Location	Nucleotide Change	Lab Change	Copy #	Mutation Type	Mutation Effect	Significance	GeneBank Acc #	Sequence Position
MSH2	Exon 2	c.1172T>C	p.S400L	1	Point mutation	Missense	Uncertain		
MSH2	Exon 2	c.590G>A	p.Arg197Ser	1	Point mutation	Missense	Benign		
MSH2	Exon 14	c.2324A>G	p.Arg742Ser	1	Point mutation	Missense	Benign		

Common Polymorphisms

Gene	Location	Nucleotide Change	Lab Change	Copy #	Mutation Type	Mutation Effect	Significance	GeneBank Acc #	Sequence Position
MSH2	Exon 2	c.1172T>C	p.S400L	1	Point mutation	Missense	Uncertain		
MSH2	Exon 2	c.590G>A	p.Arg197Ser	1	Point mutation	Missense	Benign		
MSH2	Exon 14	c.2324A>G	p.Arg742Ser	1	Point mutation	Missense	Benign		

Del/Dup

Gene	Result	Score (affected)	Single/Probe
MSH2	Negative		
MSH1	Negative		

Results Summary

Assay	Test Result	# PML1	# PML2	MSI Information	Testing Case	Test Date	MD Signature	MD Date
MSH2 Del/Dup	Negative	0	0	MSI2				
MSH2 Seq	Negative	0	1	MSI2				
MSH1 Seq	Negative	0	0	MSI2				
MSH1 Del/Dup	Negative	0	0	MSI2				

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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