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....”

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
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
Collecting Evidences

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

Courtesy of David Crockett, PhD
Collecting Evidences

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

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
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**