Panel 2: Consistency of Interpretation of Variants Across Expert Labs / Groups, ClinVar Submissions?

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Genomic Medicine VIII
June 8-9, 2015
Rockville, Maryland
Mendelian Disease Variant Classification Terminology

ACMG Recommendation:

Pathogenic (≠ mutation)
Likely pathogenic (90%)
Uncertain significance (VUS)
Likely benign
Benign (≠ polymorphism)
Defining the Challenge

83% of patients have variants that are rare or of uncertain clinical significance (5776 variants)

17% of patients have pathogenic or “likely pathogenic” variants seen ≥10 times (63 variants)

Variants of uncertain significance (71%)
Pathogenic or “likely pathogenic” variants (29%)

Figure 1. Variant Histogram from Mendelian Disease Testing of 15,000 Probands.
<table>
<thead>
<tr>
<th>Site</th>
<th>MSH6 c.2731C&gt;T; p.Arg911*</th>
<th>RYR1 c.1840C&gt;T; p.Arg614Cys</th>
<th>FBN1 c.4270C&gt;G; p.Pro1424Ala</th>
<th>TSC2 c.736A&gt;G; p.Thr246Ala</th>
<th>TNNT2 c.732G&gt;T; p.Glu244Asp</th>
<th>LDLR c.967G&gt;A; p.Gly323Ser</th>
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Amendola et al., *Genome Res* 2015. PMID: 25637381

2014 Cross-Consortium Classification of 6 Variants (early ACMG rules)
EVS 6500 Variant Classification QC: Overcalling

- Recalled all pathogenic & likely pathogenic variants:
  - 56% discordant;
  - 42/44 (95%) overcalled (final call VUS)
- Final calls matched experts
  - 142/144 (99%)

Amendola et al. *Genome Res* 2015. PMID: 25637381
ACMG STANDARDS AND GUIDELINES

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, PhD1, Nazneen Aziz, PhD2,16, Sherri Bale, PhD3, David Bick, MD4, Soma Das, PhD5, Julie Gastier-Foster, PhD6,7,8, Wayne W. Grody, MD, PhD9,10,11, Madhuri Hegde, PhD12, Elaine Lyon, PhD13, Elaine Spector, PhD14, Karl Voelkerding, MD13 and Heidi L. Rehm, PhD15; on behalf of the ACMG Laboratory Quality Assurance Committee

The American College of Medical Genetics and Genomics (ACMG) previously developed guidance for the interpretation of sequence variants. In the past decade, sequencing technology has evolved rapidly with the advent of high-throughput next-generation sequencing. By adopting and leveraging next-generation sequencing, clinical laboratories are now performing an ever-increasing catalogue of genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. By virtue of increased complexity, this shift in genetic testing has been accompanied by new challenges in sequence interpretation. In this context the ACMG convened a workgroup in 2013 comprising representatives from the ACMG, the Association for Molecular Pathology (AMP), and the College of American Pathologists to revisit and revise the standards and guidelines for the interpretation of sequence variants. The group consisted of clinical laboratory directors and clinicians. This report represents expert opinion of the workgroup with input from ACMG, AMP, and College of American Pathologists stakeholders. These recommendations primarily apply to the breadth of genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. This report recommends the use of specific standard terminology—“pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”—to describe variants identified in genes that cause Mendelian disorders. Moreover, this recommendation describes a process for classifying variants into these five categories based on criteria using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data). Because of the increased complexity of analysis and interpretation of clinical genetic testing described in this report, the ACMG strongly recommends that clinical molecular genetic testing should be performed in a Clinical Laboratory Improvement Amendments–approved laboratory, with results interpreted by a board-certified clinical molecular geneticist or molecular genetic pathologist or the equivalent.

Genet Med advance online publication 5 March 2015

Key Words: ACMG laboratory guideline; clinical genetic testing; interpretation; reporting; sequence variant terminology; variant reporting
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ACMG Standard Recs
Richards et al GIM 2015
PMID:25741868
ACMG Variant Classification Rules, continued

2015 CSER “bakeoff”

99 germline variants
-9 classified by 9 sites
-90 classified by 2-3 sites
by ACMG and own rules
### Intra-laboratory Usual vs. ACMG Classification Comparison

9 labs x 9 variants

<table>
<thead>
<tr>
<th>ACMG class</th>
<th>P</th>
<th>LP</th>
<th>VUS</th>
<th>LB</th>
<th>B</th>
<th>Total</th>
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<td>10</td>
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<tr>
<td><strong>Total</strong></td>
<td>16</td>
<td>21</td>
<td>19</td>
<td>17</td>
<td>8</td>
<td>81</td>
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- 73% concordant
- 9% ACMG less pathogenic
- 19% ACMG more pathogenic

If discordant, ACMG less certain 77% (e.g. VUS; blue boxes; 17/22)
Intra-laboratory Usual vs. ACMG Classification Comparison:
98 variants (90 average 2.85 calls, 9 have 9 calls)

- 268/335 (80%) concordant; 12/335 (3.6%) shift by >1 class
- 26/335 (7.8%) ACMG less pathogenic
- 41/335 (12.2%) ACMG more pathogenic

If discordant, ACMG less certain (e.g. VUS) 45/67 (67%)
Labs call more things benign, likely benign.

<table>
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<tr>
<th>Lab class</th>
<th>P</th>
<th>LP</th>
<th>VUS</th>
<th>LB</th>
<th>B</th>
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<td>VUS</td>
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<td>4</td>
<td>5</td>
<td>28</td>
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<td><strong>Total</strong></td>
<td>70</td>
<td>74</td>
<td>119</td>
<td>40</td>
<td>32</td>
<td>335</td>
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- MAF > 5%
- MAF > disease frequency

Benign
(i) 1 Stand-alone (BA1) OR
(ii) ≥2 Strong (BS1–BS4)

Likely benign
(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) OR
(ii) ≥2 Supporting (BP1–BP7)

Uncertain significance
(i) Other criteria shown above are not met OR
(ii) the criteria for benign and pathogenic are contradictory
Inter-laboratory Concordance of 98 variants

Count

BETTER

Difference in classifications across labs

- All labs agree
- 1 off
- 2 off
- 3 off
- 4 off

Benign to pathogenic

P=0.9

ACMG criteria
Lab criteria

1. Pathogenic
2. Likely pathogenic
3. VUS
4. Likely benign
5. Benign
Variant with Major Disagreement: Why?

**SPG7:c.1529C>T (p.Ala510Val)**
- 0.4% EU chromosomes (267/66688; 0.8% people; ExAC); 3/50 people in CSER
- AR, late-onset, +/- reduced penetrance, spastic paraplegia

**Laboratory classification**
- AR, trans
- Cosegregation
- Computational

**ACMG Classification**
- Time: 25 (LB/VUS) to >200 (VUS/P) minutes
- MAF > disease frequency

<table>
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<th>ACMG Rules</th>
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<th>PM3</th>
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ACMG lines of evidence:
- **Pathogenic**: PS1, PS3, PM3, PP1, PP3, PP5
- **Likely Pathogenic**: PS1, PS3(moderate), PS4, PM3, PP1, PP3
- **Pathogenic (strong)**: PS1, PS3, PM3, PP1, PP3
- **Uncertain Significance**: PS1, PS3, PS4, BS1
- **Likely Benign**: PS1, PS3, PS4, BS1
- **Uncertain Significance**: PS1, PS3, PS4, BS1

Relevant evidence:
- MAF > disease frequency
- AR, trans
- Cosegregation
- Functional evidence
- Computational
Sample size to determine pathogenicity

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<th>MAF = 0.001%</th>
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<td>RR=1.5</td>
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</table>

- If population-based cohort, large number to get different types of disease covered.
- Some variants will occur only in some ancestry groups.

Shirts et al. GIM 2014
PMID:24357849
The FDA and Genomic Tests — Getting Regulation Right
Barbara J. Evans, Ph.D., J.D., Wylie Burke, M.D., Ph.D., and Gail P. Jarvik, M.D., Ph.D.

The Food and Drug Administration (FDA) recently advanced two draft guidances\(^1\),\(^2\) proposing a regulatory framework for laboratory-developed tests, a category that includes many but not all genomic tests. The FDA convened a workshop in February 2015 to discuss the oversight of next-generation sequencing.\(^3\),\(^4\) President Barack Obama’s Precision Medicine Initiative calls for the FDA to modernize its approach to genomic testing\(^5\),\(^6\) as a ment discretion policy that shields many laboratory-developed tests from being regulated as medical devices.\(^1\),\(^2\) The agency believes its “policy of general enforcement discretion” for laboratory-developed tests “is no longer appropriate” in light of profound changes in technology and business practices. This raises a question: Are the FDA medical device regulations also out of date? These regulations rely heavily on statutory
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ClinVar: ClinGen’s Variant Repository

Researchers
Clinical Labs
Expert Groups

Unpublished or Literature Citations

ClinVar
Variant-level Data

Sharing Clinical Reports Project
Genome Connect and Free-the-Data

Patients

Clinics

Linked Databases
OMIM
CFTR2
BIC
InSiGHT

Patient Registries

>315 ClinVar submitters
>172,000 submissions
>118,000 unique interpreted variants
Assertion Levels in ClinVar

- Practice Guideline
- Expert Panel
- Multi-Source Consistency
- Single Submitter – Criteria Provided
- Single Submitter – No Criteria Provided
- No Assertion

- ACMG, CPIC  
- CFTR2, InSiGHT, PharmGKB, ENIGMA

No Assertion Not applicable

Distinction Launching in June
ClinVar Variant Database

11% (12,895/118,169) of variants have ≥2 submitters in ClinVar

17% (2229/12,895) are interpreted differently

ClinVar Data from May 4th, 2015
Main reasons for discrepancies was variant classification rules
- Novel silent: LB vs VUS
- Missense (freq cut-offs; MOI)

Work of:
Birgit Funke
Steven Harrison
Melissa Kelly
Lori Bean
Amy Knight
Madhuri Hegde
Supporting a Curation Environment for both Crowd-Sourcing and Expert Consensus

ClinVar

ClinGenKB

Case-level data store

Machine-learning algorithms

Data resources

Cardiovascular Disease WG

Inborn Errors of Metabolism WG

Hereditary Cancer WG

PGx WG

Somatic Cancer WG

Unpublished or Literature Citations

Expert Curated Variants

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<table>
<thead>
<tr>
<th>Data Type</th>
<th>Supporting (BS)</th>
<th>Moderate (PP)</th>
<th>Strong (PS)</th>
<th>Very Strong (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population Data</strong></td>
<td>MAF frequency is too high for disorder OR observation in controls inconsistent with disease penetrance BS2</td>
<td>Multiple lines of computational evidence suggest no impact on gene /gene product BP4</td>
<td>Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5</td>
<td>Same amino acid change as an established pathogenic variant PS1</td>
</tr>
<tr>
<td>Computational And Predictive Data</td>
<td>Missense in gene where only truncating cause disease BP1</td>
<td>Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP2</td>
<td>Located in a mutational hot spot and/or known functional domain PM1</td>
<td>Truncating variant in a gene where LOF is a known mechanism of disease PVS1</td>
</tr>
<tr>
<td><strong>Functional Data</strong></td>
<td>Well-established functional studies show no deleterious effect BS3</td>
<td>In-frame indels in a repetitive region without a known function BP3</td>
<td>Missense in gene with low rate of benign missense variants and path. missenses common PP2</td>
<td>Increased segregation data</td>
</tr>
<tr>
<td>Segregation Data</td>
<td>Non-segregation with disease BS4</td>
<td>Co-segregation with disease in multiple affected family members PP1</td>
<td>De novo (without paternity &amp; maternity confirmed) PM6</td>
<td>De novo (paternity &amp; maternity confirmed) PS2</td>
</tr>
<tr>
<td>De novo Data</td>
<td>Observed in trans with a dominant variant BP2</td>
<td>For recessive disorders, detected in trans with a pathogenic variant PM3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic Data</td>
<td>Observed in cis with a pathogenic variant BP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Database</td>
<td>Reputable source = benign BP6</td>
<td>Reputable source = pathogenic PP5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Data</td>
<td>Found in case with an alternate cause BP5</td>
<td>Patient’s phenotype or FH highly specific for gene PP4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Need tool/resource Quantifiable**
Major Clinical Domain WG Charges

• Define the *genes* with valid association to a human disease

• Define *variants* with valid evidence for pathogenicity and those with benign impact

• Define *rules* for interpreting *novel* variants
The two axes of implication

- VUS in GUS
- VUS in BRCA1 gene
- Phe508del in CFTR
- Can’t exist

Modified from Daniel MacArthur
<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive</td>
<td>Repeatedly demonstrated in research &amp; clinical settings.</td>
</tr>
<tr>
<td>Strong</td>
<td>Excess of pathogenic variants in cases vs. controls &amp; supporting experimental data.</td>
</tr>
<tr>
<td>Moderate</td>
<td>≥3 unrelated probands with pathogenic variants &amp; supporting experimental data.</td>
</tr>
<tr>
<td>Limited</td>
<td>&lt;3 probands w/ pathogenic variants.</td>
</tr>
<tr>
<td>No Evidence Reported</td>
<td>“Candidate” genes based on animal models or disease pathways, but no pathogenic variants reported.</td>
</tr>
<tr>
<td>Disputed</td>
<td>Significant evidence <em>refuting</em> a role for gene in this disease.</td>
</tr>
<tr>
<td>Evidence Against</td>
<td>Evidence refuting the role of the gene significantly outweighs any supporting evidence.</td>
</tr>
</tbody>
</table>

Application of ClinGen Gene-Disease Evidence Rules

Pheo/Para (19 Genes)
Hearing Loss (91 Genes)
BabySeq (1504 Genes)

Definitive
Strong
Moderate
Limited/Disputed
Proposed Evidence Required to Include a Gene In a Clinical Test:

- Definitive evidence
- Strong evidence
- Moderate evidence
- Limited evidence
- Disputed evidence

Genes with less evidence can be included in test design and analyzed in a research context to build evidence.
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The Stakes are High in the Clinical Application of Genomics

 Patients (& families) make serious decisions. False positives lead to:

- Unnecessary surgery; years of unnecessary screening
- Premature end to diagnostic pursuit, forgoing the true answer

- False negatives lead to:
  - Forgoing necessary preventive/therapeutic modalities
  - Amplified by misclassification of family members as at-risk or not
  - Family planning & abortion
  - The psychological damage of misinformation
Open Discussion
1. Critical Knowledge Gaps Impeding Genomic Medicine Implementation

• 21% of variants in ClinVar are VUSs and 17% are interpreted differently

• Case-level knowledge and other evidence is not being collected in ClinVar

2. Other Key Barriers to Implementation

• Use of inconsistent systems/implementations for evaluating variants (evidence assessment and interpretation)

• Cost and complexity of building support for variant assessment is difficult for laboratories to take on
3. Recommended Approaches to Addressing Gaps and Barriers

• Build and continue to iterate on a tool to support variant assessment - ClinGen work in progress

• need web-based environment for collaborative curation with access to all evidence (Wiki-like)

• Tool should be open source to allow download and integration into laboratory workflows (structured data shared back into web-based environment)

• Tool should provide easy access to data and support for rule usage

• Need publication process to require submission of interpreted variants to ClinVar and supporting evidence (e.g. case-level data) into accessible databases to support curation

• Need to integrate electronic systems capturing case-level evidence (e.g. clinical laboratory DBs, EHRs, research study DBs) into an accessible federated network
3. Training Needs and Approaches

• Need to ensure **consistent training in variant assessment**
  ✓ Incorporate into all training programs (medical school, graduate school in biological disciplines, postdoctoral studies in genomics, residency programs in medical genetics, fellowships in laboratory genetics, genetic counseling programs)

• Need **training of healthcare providers** on how to use genetic information of “likely” or uncertain significance and evaluate quality of source of interpretations (e.g. expert or single opinion)
  ✓ Continuing education of healthcare providers
  ✓ Guidelines in specific clinical disciplines
5. Bedside Back to Bench Research Questions: Facilitating a Virtuous Cycle

• Need **higher throughput approaches** to assess the impact of human variation – feed all VUSs back into research studies

• Identification of **candidate genes** from clinical WES needs to feed into research studies (e.g. matchmaker exchange)

• **Collection of clinical cases** with known genetic disorders to define targeted population for deeper studies and clinical trials

• Need return of results process to integrate back into learning system (**collect outcomes and rephenotyping**)  
  - Examples:
    - Unaffected family tests negative for familial variant and later develops the disease
    - Genetic results suggest specific treatment – did it work? – need to collect outcomes