Community-Driven Approaches to Support Variant Interpretation

Steven M. Harrison, PhD

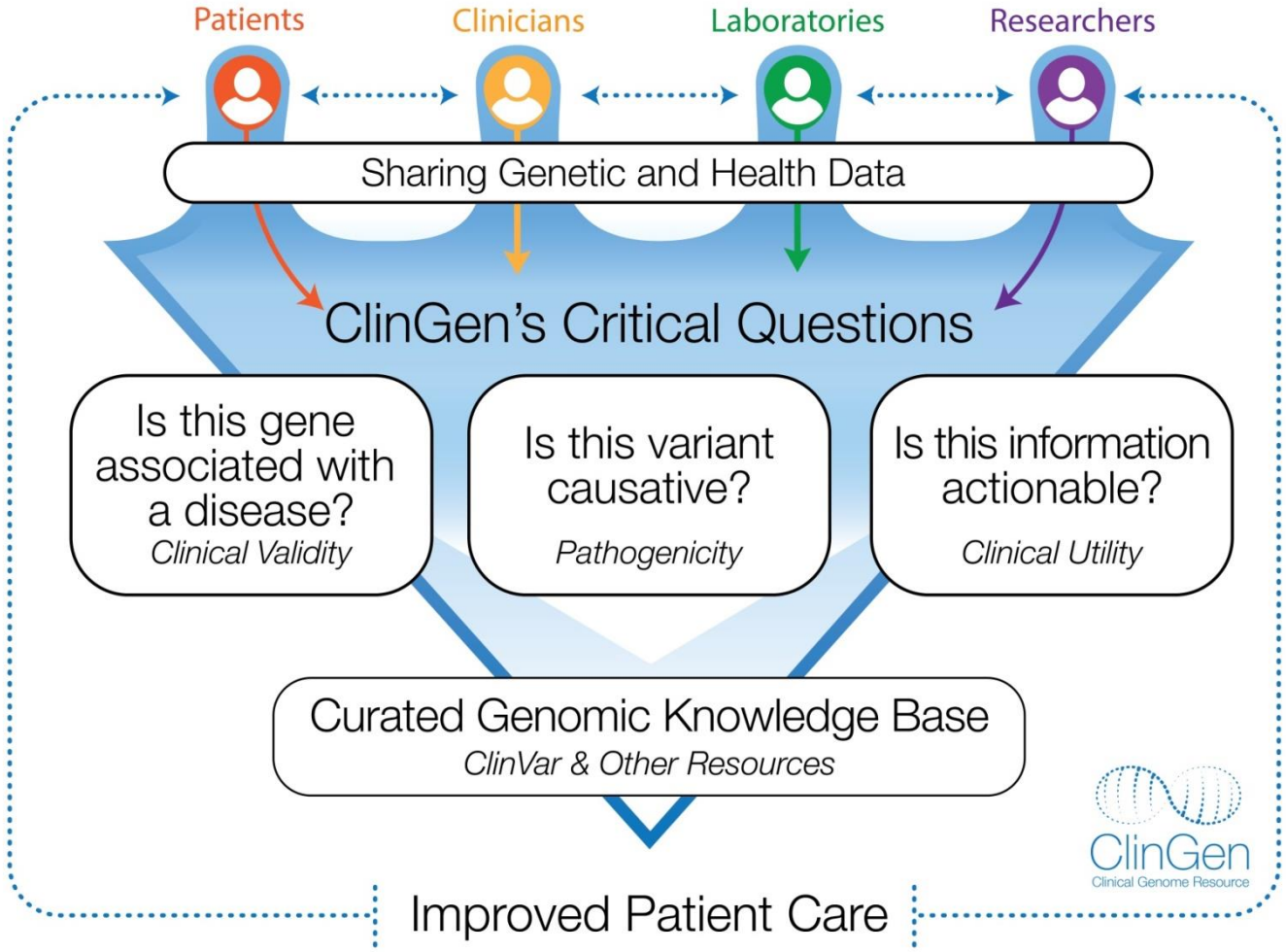
Variant Scientist, Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine; Harvard Medical School

PARTNERS HEALTHCARE | PERSONALIZED MEDICINE

HARVARD MEDICAL SCHOOL

The Clinical Genome Resource

Purpose: Create authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research.



Rehm *et al.* ClinGen - The Clinical Genome Resource. N Engl J Med 2015; 372:2235-2242

www.clinicalgenome.org

>400 people from >90 institutions

ClinGen Acknowledgements

ClinGen Steering Committee

Jonathan Berg, UNC
Lisa Brooks, NHGRI
Carlos Bustamante, Stanford
Mike Cherry, Stanford
James Evans, UNC
Andy Faucett, Geisinger
Katrina Goddard, Kaiser Permanente
Danuta Krotoski, NICHD
Melissa Landrum, NCBI
David Ledbetter, Geisinger
Christa Lese Martin, Geisinger
Aleks Milosavljevic, Baylor
Robert Nussbaum, UCSF
Kelly Ormond, Stanford
Sharon Plon, Baylor
Erin Ramos, NHGRI
Heidi Rehm, Harvard
Sheri Schully, NCI
Steve Sherry, NCBI
Michael Watson, ACMG
Kirk Wilhelmsen, UNC
Marc Williams, Geisinger

Program Coordinators:
Danielle Azzariti, Brianne Kirkpatrick, Kristy Lee, Laura Milko, Annie Niehaus, Misha Rashkin, Erin Riggs, Andy Rivera, Cody Sam, Yekaterina Vaydylevich, Meredith Weaver

ClinGen Working Groups (WG)

Genomic Variant WG

Chairs: Christa Martin, Sharon Plon, Heidi Rehm

Sequence Variant Interpretation WG

Chairs: Les Beisecker, Marc Greenblat

Phenotyping WG

Chair: David Miller

ClinVar IT Standards and Data Submission WG

Chair: Karen Eilbeck, Melissa Landrum

Data Model WG

Chairs: Larry Babb, Chris Bizon

Informatics WG

Chair: Carlos Bustamante

Clinical Domain WGs

Hereditary Cancer: Matthew Ferber, Ken Offit, Sharon Plon

Somatic Cancer: Shashi Kulkarni, Subha Madhavan

Cardiovascular: Euan Ashley, Birgit Funke, Ray Hershberger

Metabolic: Rong Mao, Robert Steiner, Bill Craigen

Pharmacogenomic: Teri Klein, Howard McLeod

Education, Engagement, Access WG

Chairs: Andy Faucett, Erin Riggs

Consent and Disclosure Recommendations (CADRe) WG

Chairs: Andy Faucett, Kelly Ormond

Gene Curation WG

Chairs: Jonathan Berg, Christa Martin

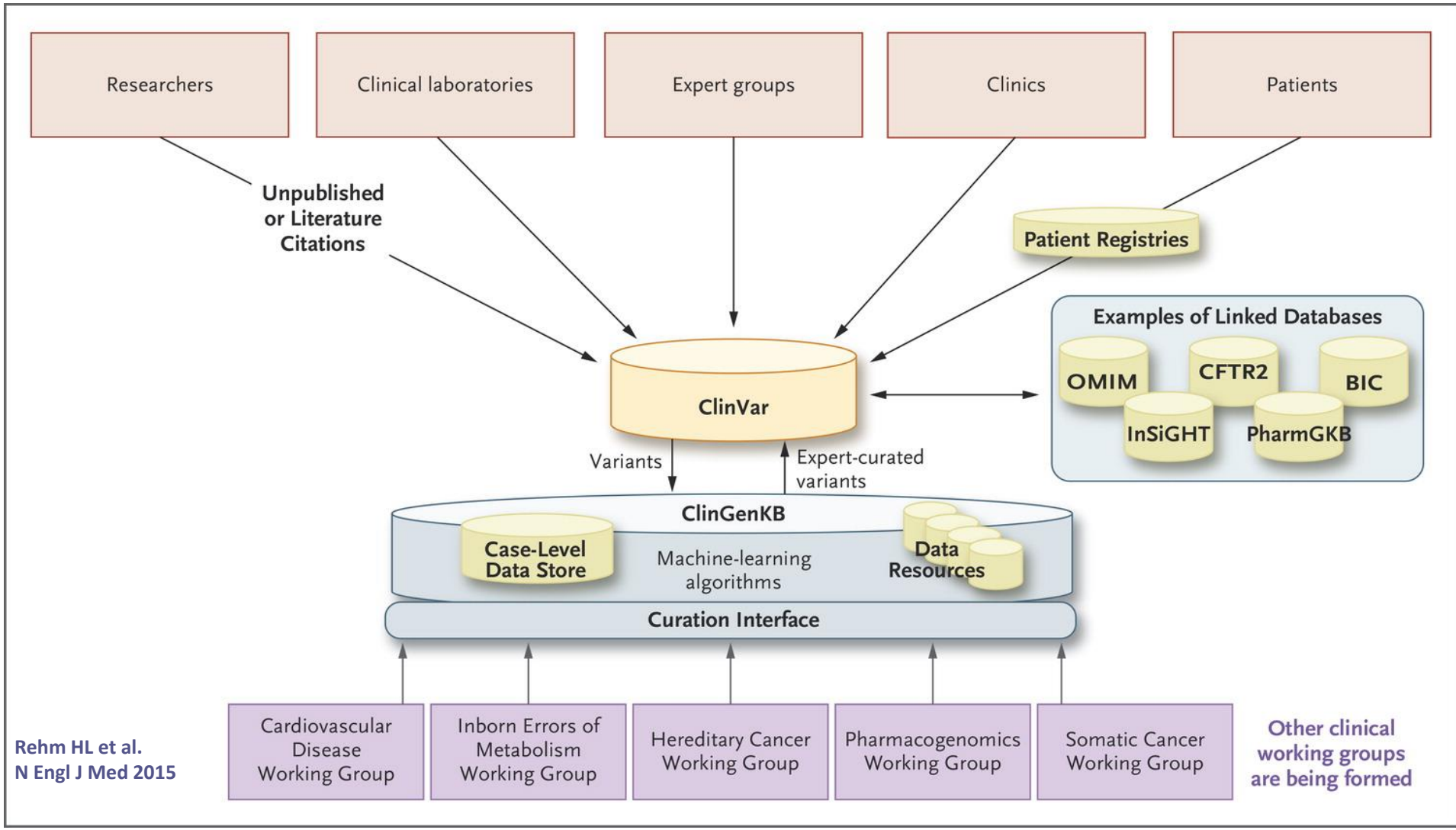
Actionability WG

Chairs: Jim Evans, Katrina Goddard

EHR WG

Chair: Marc Williams

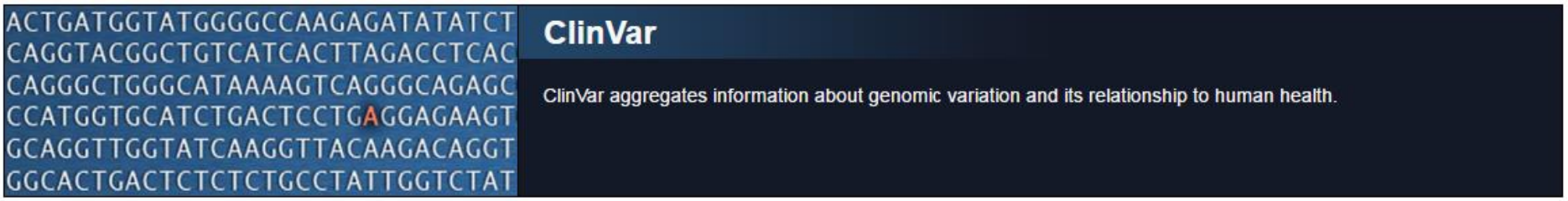
Follow of Data through ClinGen



Rehm HL et al.
N Engl J Med 2015

ClinGen Efforts to Support Variant Interpretation

- ClinVar
  - Assertion criteria
  - Variant review status
- Interpretation discrepancies
  - How to identify discrepancies
  - Resolution attempts and progress
- ClinGen Disease Area ACMG specification
  - RASopathy
  - Cardiomyopathy / MYH7
- ClinGen Gene Curation

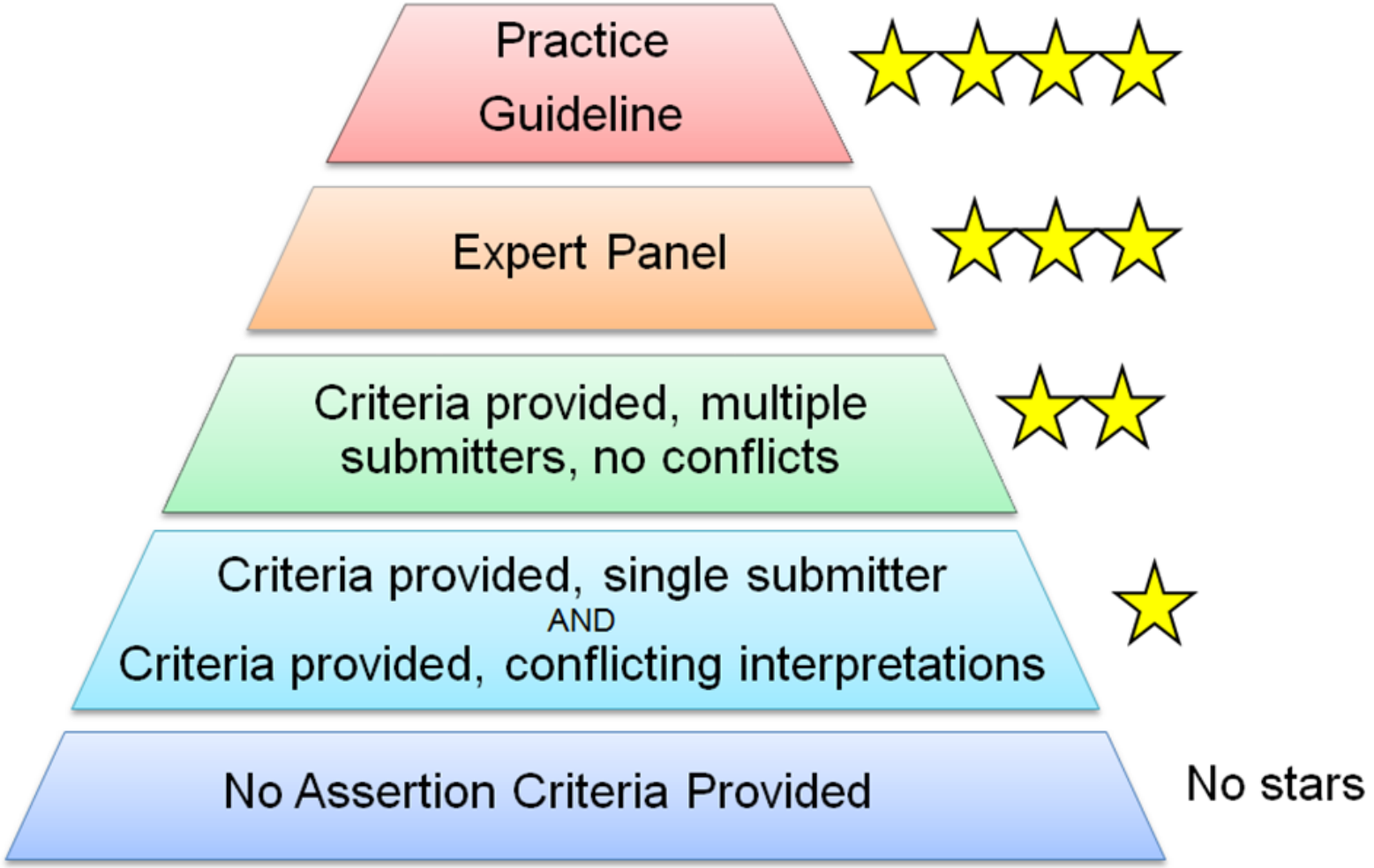


- Public archive of reports of the relationships among genomic variants and phenotypes.

- ClinVar aggregates submissions of the same variant and determines if the submitted clinical interpretations are **conflicting or concordant**

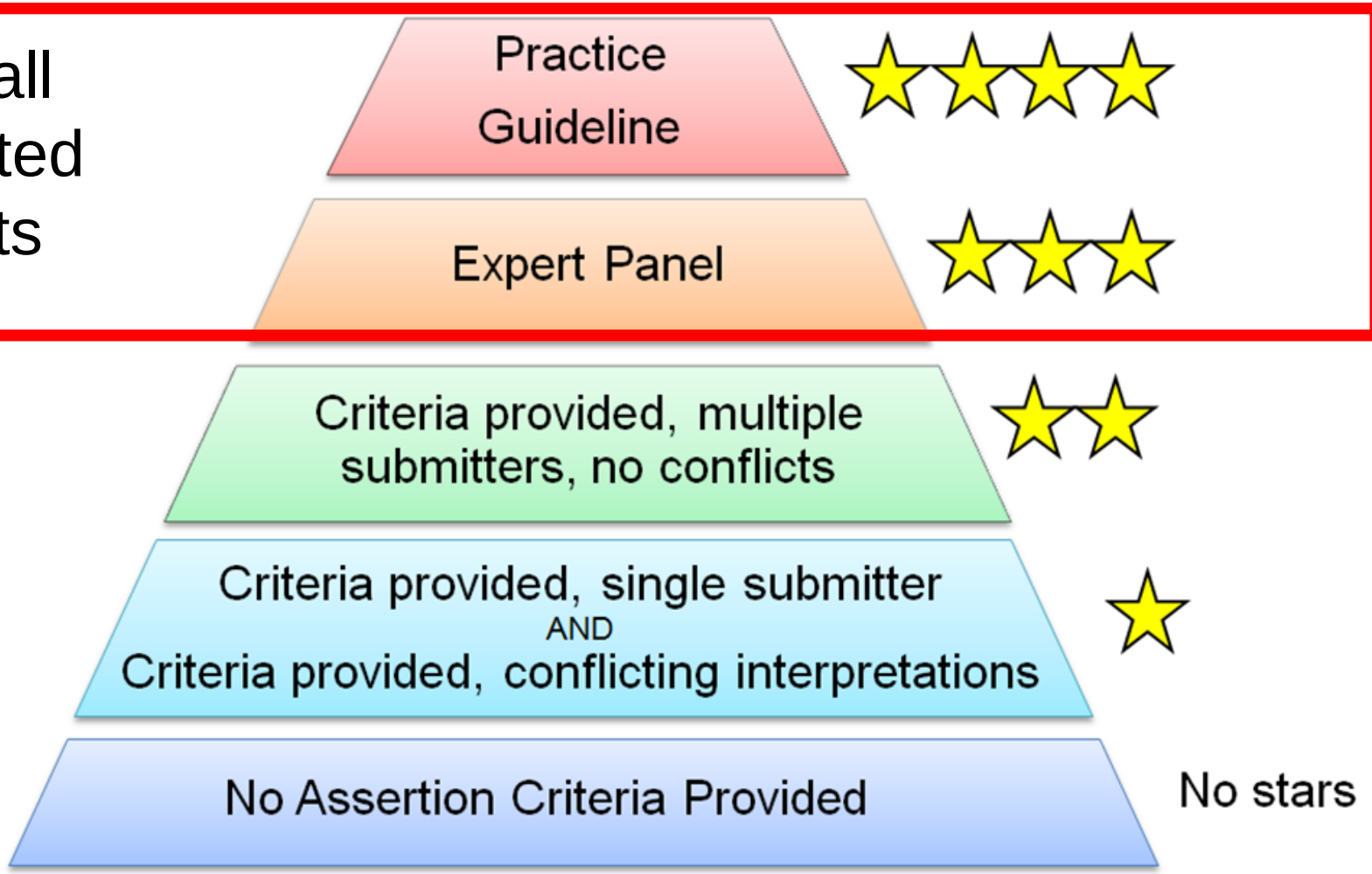
- Currently **139,791** unique variants represented (3/28/16)
  - **126,247** variants with interpretations (90%)

ClinGen developed a tiered rating system to designate the review level of each variant in ClinVar



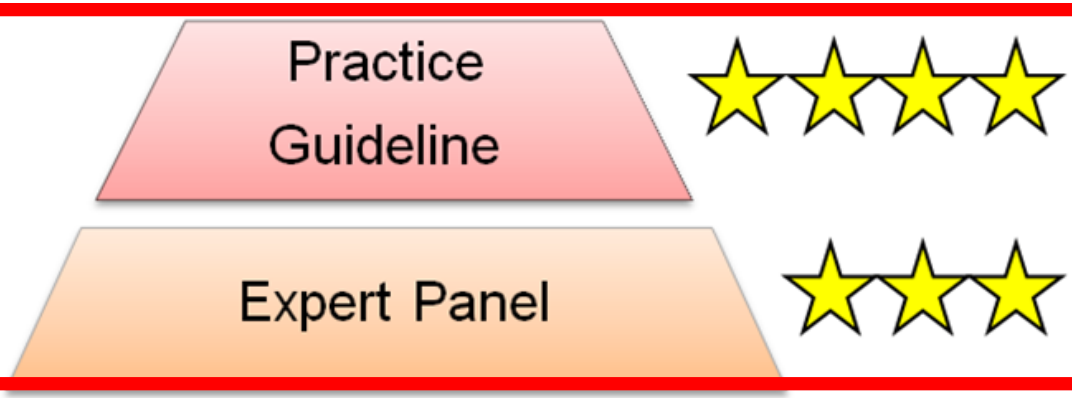
ClinGen developed a tiered rating system to designate the review level of each variant in ClinVar

3% of all interpreted variants

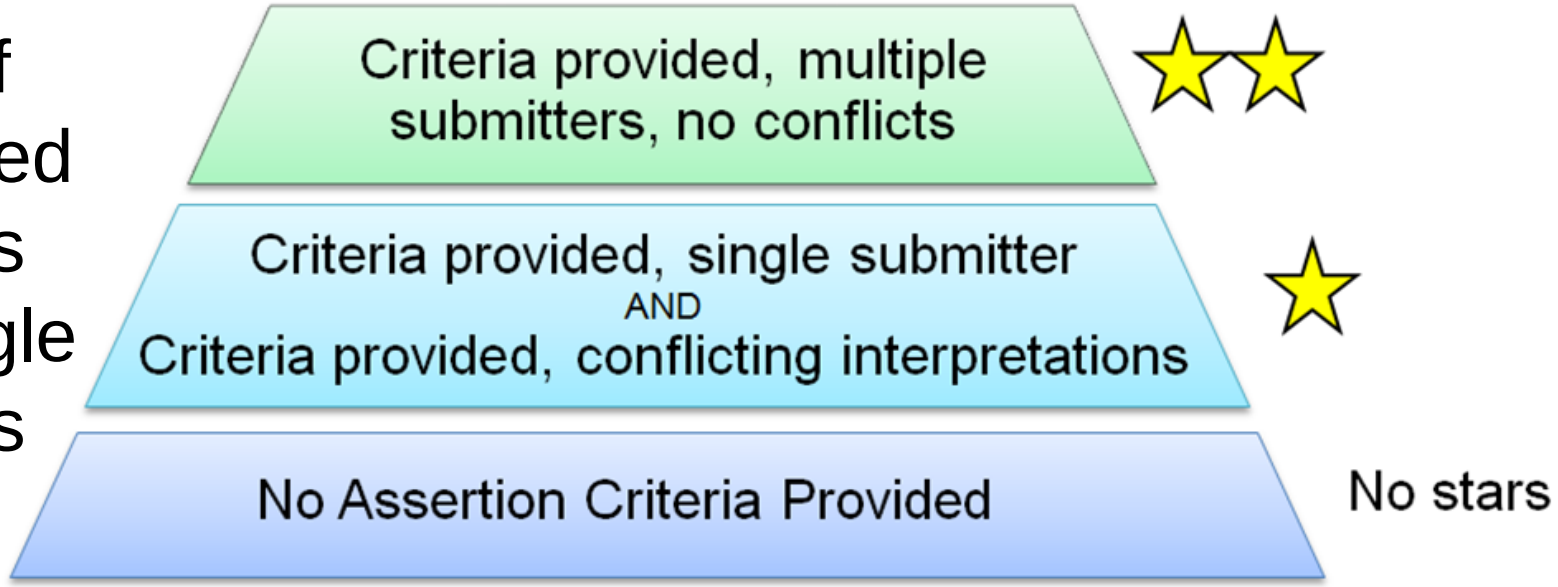


ClinGen developed a tiered rating system to designate the review level of each variant in ClinVar

3% of all interpreted variants



97% of interpreted variants from single sources



Assertion Criteria

ClinVar acknowledges and more heavily weights interpretations from submitters who attest to certain approaches and provide documentation of methods

1. Attest to a comprehensive review of variant evidence
2. Use a scoring system with at least 3 levels (e.g. pathogenic, uncertain significance, benign for Mendelian disease variants)
3. Share criteria used to assign a variant to each category
4. Inclusion of supporting evidence or a rationale for the classification of variants and/or willingness to be contacted by ClinVar users to provide supporting evidence.

NM_007294.3(BRCA1):c.5363G>T (p.Gly1788Val)

Variation ID: 37660
Review status: ★ ★ ★ ☆ reviewed by expert panel

Interpretation

Go to:

Clinical significance: Pathogenic
Last evaluated: Aug 10, 2015
Number of submission(s): 5
Condition(s):
- Familial cancer of breast [MedGen - OMIM]
- Breast-ovarian cancer, familial 1 [MedGen - OMIM]
- Hereditary cancer-predisposing syndrome [MedGen]

Assertion and evidence details

Go to:

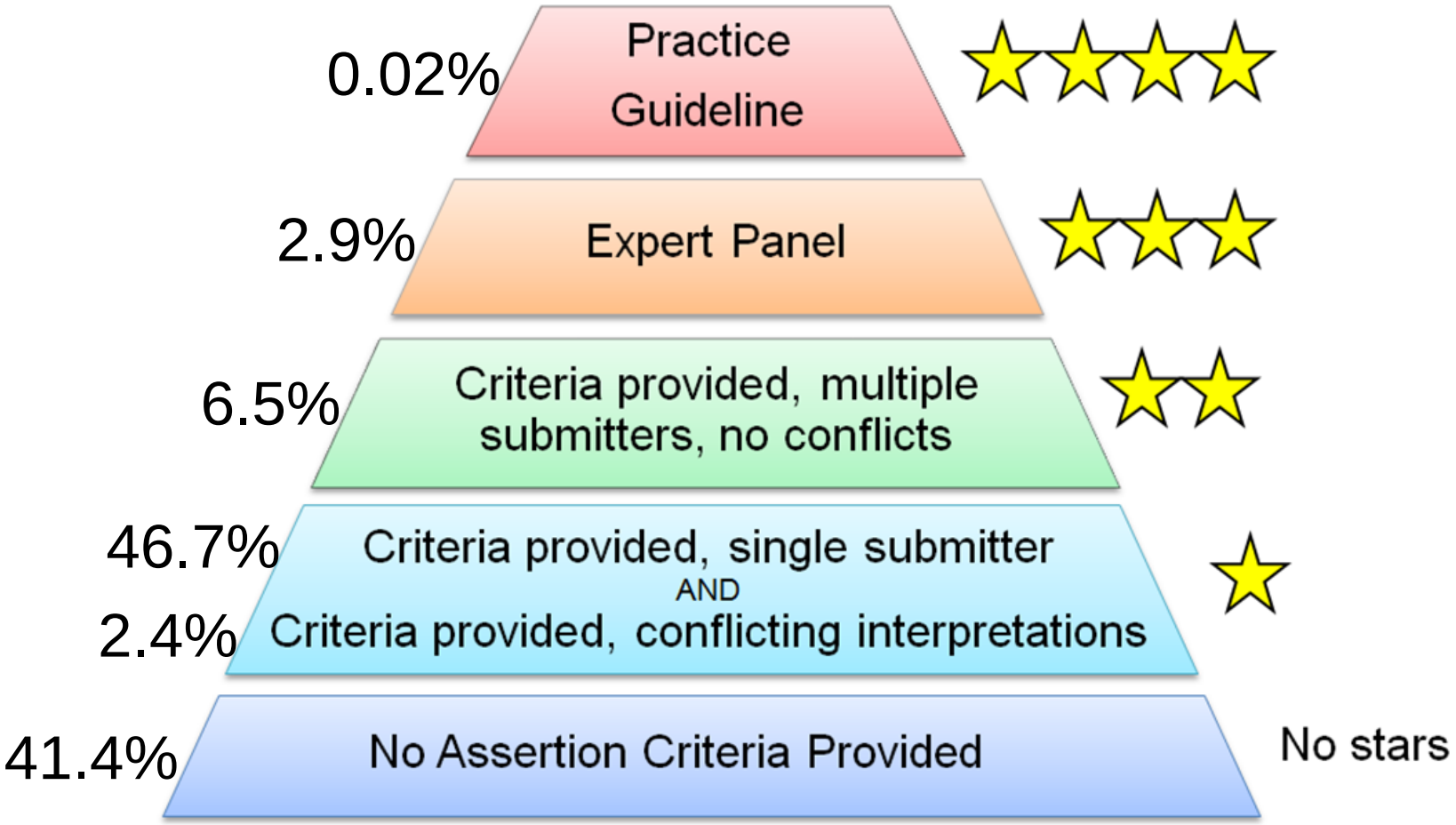
Clinical assertions | Summary evidence | Supporting observations

Germline

Filter:

Clinical significance (Last evaluated)	Review status (Assertion method)	Collection method	Condition(s) (Mode of inheritance)	Origin	Citations	Submitter - Study name (Last submitted)	Submission accession
Pathogenic (Aug 10, 2015)	reviewed by expert panel (ENIGMA BRCA1/2 Classification Criteria (2015))	curation	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline	PubMed (1) [See all records that cite this PMID] Other citation	Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) Study description (Aug 17, 2015)	SCV000244400.1
Pathogenic (Oct 11, 2014)	criteria provided, single submitter (Ambry Autosomal Dominant and X-Linked criteria (9/4/14))	clinical testing	Hereditary cancer-predisposing syndrome [MedGen]	germline		Ambry Genetics (Feb 13, 2015)	SCV000213372.1
Pathogenic (May 29, 2002)	no assertion criteria provided	clinical testing	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline		Breast Cancer Information Core (BIC) (BRCA1) (Mar 28, 2014)	SCV000145482.1
Pathogenic (Sep 7, 2011)	no assertion criteria provided	clinical testing	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline		Sharing Clinical Reports Project (SCRP) (Dec 30, 2013)	SCV000053845.4
not provided (Feb 1, 2013)	no assertion provided	literature only	Familial cancer of breast [MedGen | OMIM]	germline	PubMed (4) [See all records that cite these PMIDs]	Invitae (Mar 30, 2013)	SCV000076974.2

ClinVar Variant Review Levels



NM_020632.2(ATP6V0A4):c.1739T>C (p.Met580Thr)

Clinical significance: **Pathogenic**

Review Status: (0/4) no assertion criteria provided

Clinical significance (Last evaluated)	Review status (Assertion method)	Collection method	Condition(s) (Mode of inheritance)	Origin	Citations	Submitter - Study name (Last submitted)	Submission accession
Pathogenic (Sep 1, 2000)	no assertion criteria provided	literature only	Renal tubular acidosis, distal, autosomal recessive [MedGen | OMIM]	germline	PubMed (1) [See all records that cite this PMID]	OMIM (Dec 30, 2010)	SCV000025643.2

NM_007294.3(BRCA1):c.5363G>T (p.Gly1788Val)

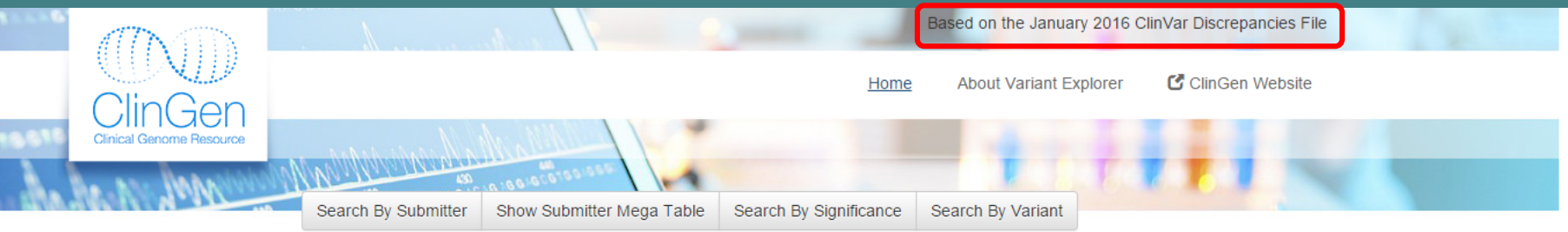
Clinical significance: **Pathogenic**

Review Status: (3/4) Reviewed by expert panel

Clinical significance (Last evaluated)	Review status (Assertion method)	Collection method	Condition(s) (Mode of inheritance)	Origin	Citations	Submitter - Study name (Last submitted)	Submission accession
Pathogenic (Aug 10, 2015)	reviewed by expert panel (ENIGMA BRCA1/2 Classification Criteria (2015))	curation	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline	PubMed (1) [See all records that cite this PMID] Other citation	Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) Study description (Aug 17, 2015)	SCV000244400.1
Pathogenic (Oct 11, 2014)	criteria provided, single submitter (Ambry Autosomal Dominant and X-Linked criteria (9/4/14))	clinical testing	Hereditary cancer-predisposing syndrome [MedGen]	germline		Ambry Genetics (Feb 13, 2015)	SCV000213372.1
Pathogenic (May 29, 2002)	no assertion criteria provided	clinical testing	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline		Breast Cancer Information Core (BIC) (BRCA1) (Mar 28, 2014)	SCV000145482.1
Pathogenic (Sep 7, 2011)	no assertion criteria provided	clinical testing	Breast-ovarian cancer, familial 1 [MedGen | OMIM]	germline		Sharing Clinical Reports Project (SCRP) (Dec 30, 2013)	SCV000053845.4
not provided (Feb 1, 2013)	no assertion provided	literature only	Familial cancer of breast [MedGen | OMIM]	germline	PubMed (4) [See all records that cite these PMIDs]	Invitae (Mar 30, 2013)	SCV000076974.2

Interpretation Discrepancy Identification and Resolution

- Identify interpretation differences
  - Monthly ClinVar report
    - Available on FTP site (updated monthly)
  - VariantExplorer.org
- Resolution process with ClinGen



Welcome to VariantExplorer!

The goal of VariantExplorer is to facilitate identification of clinical significance interpretation discrepancies in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), a submitter-driven repository that archives reports of the relationships among genomic variants and phenotypes submitted by clinical laboratories, researchers, clinicians, expert panels, practice guidelines, and other groups or organizations. Given the large number of submitters to ClinVar, many variants have interpretations from multiple submitters and those interpretations may not always agree.

By displaying how the full set of variant interpretations from a specific submitter compares to all other submitters (or to another specific submitter), VariantExplorer helps users view the types and levels of discrepancies in ClinVar. The submitter-specific Clinical Significance Breakdown Tables (seen below) displays pair-wise counts of discrepant interpretations, including confidence discrepancies (such as Benign vs Likely benign or Pathogenic vs Likely pathogenic). For example, the table below indicates there are 12 variants in ClinVar interpreted as Likely benign by Submitter A and interpreted as Uncertain significance by Submitter B. By displaying the discrepancies in this manner, VariantExplorer hopes to facilitate resolution of interpretation discrepancies.

Clinical Significance Breakdown Table example

	CLINVAR SUBMITTER B		
CLINVAR SUBMITTER A	Clinical significance	Pathogenic	Likely pathogenic
	Pathogenic	0	5
	Likely pathogenic	2	0
	Uncertain significnace	1	1
	Likely benign	0	0
	Benign	0	0

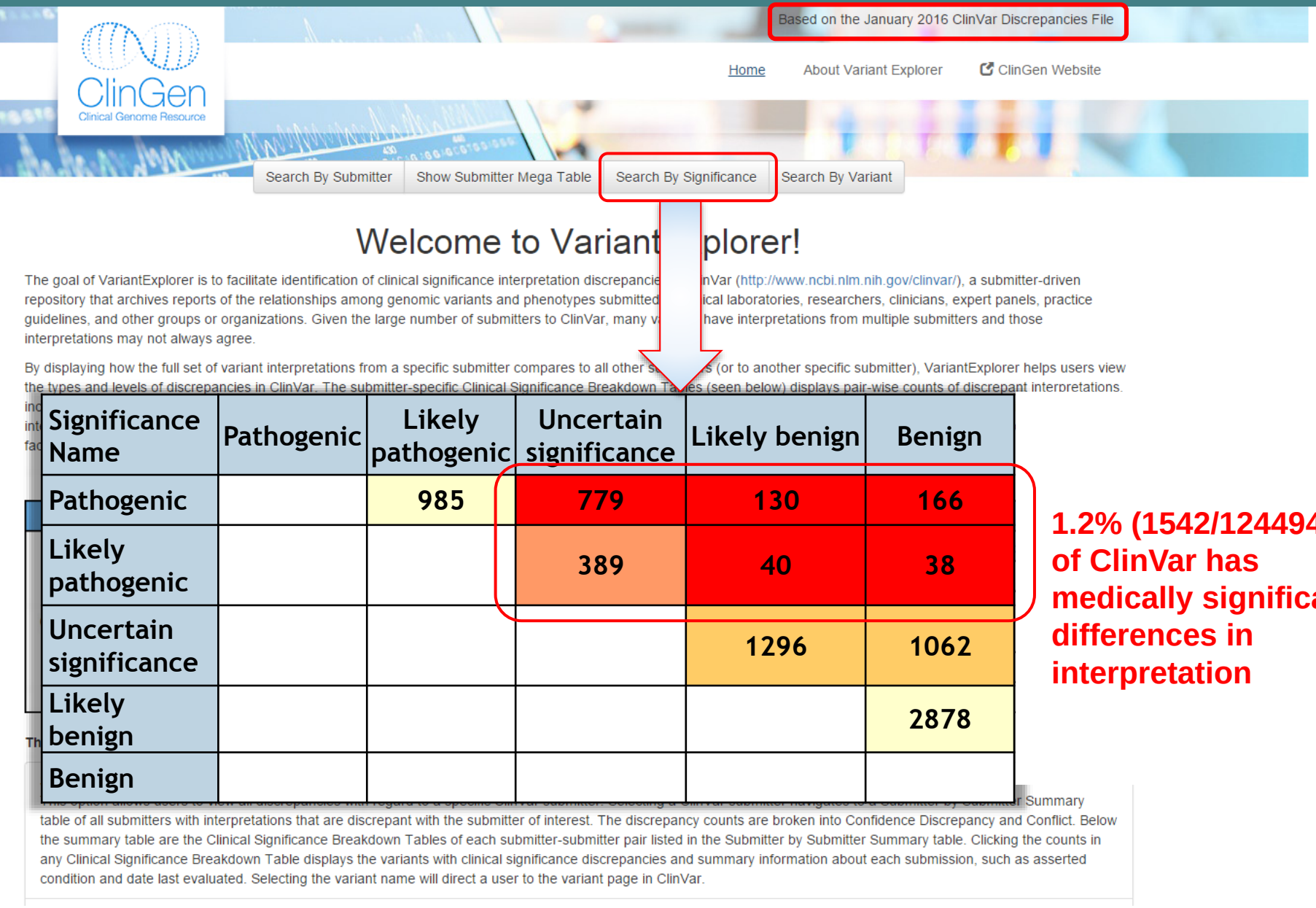
The discrepancy data in VariantExplorer can be viewed from four different approaches:

Search By Submitter

This option allows users to view all discrepancies with regard to a specific ClinVar submitter. Selecting a ClinVar submitter navigates to a Submitter by Submitter Summary table of all submitters with interpretations that are discrepant with the submitter of interest. The discrepancy counts are broken into Confidence Discrepancy and Conflict. Below the summary table are the Clinical Significance Breakdown Tables of each submitter-submitter pair listed in the Submitter by Submitter Summary table. Clicking the counts in any Clinical Significance Breakdown Table displays the variants with clinical significance discrepancies and summary information about each submission, such as asserted condition and date last evaluated. Selecting the variant name will direct a user to the variant page in ClinVar.

variantexplorer.org

Justin Aronson, Steven Harrison, Larry Babb, Sandy Aronson, Heidi Rehm

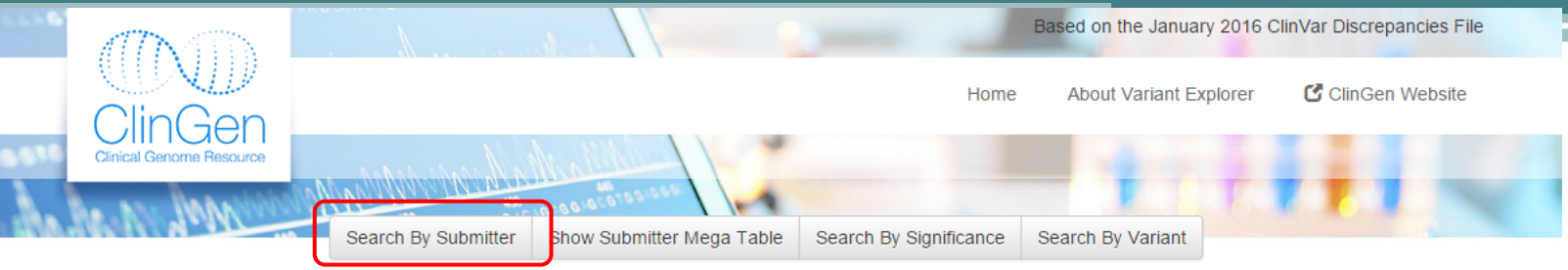


Significance Name	Pathogenic	Likely pathogenic	Uncertain significance	Likely benign	Benign
Pathogenic		985	779	130	166
Likely pathogenic			389	40	38
Uncertain significance				1296	1062
Likely benign					2878
Benign					

1.2% (1542/124494) of ClinVar has medically significant differences in interpretation

variantexplorer.org

Justin Aronson, Steven Harrison, Larry Babb, Sandy Aronson, Heidi Rehm



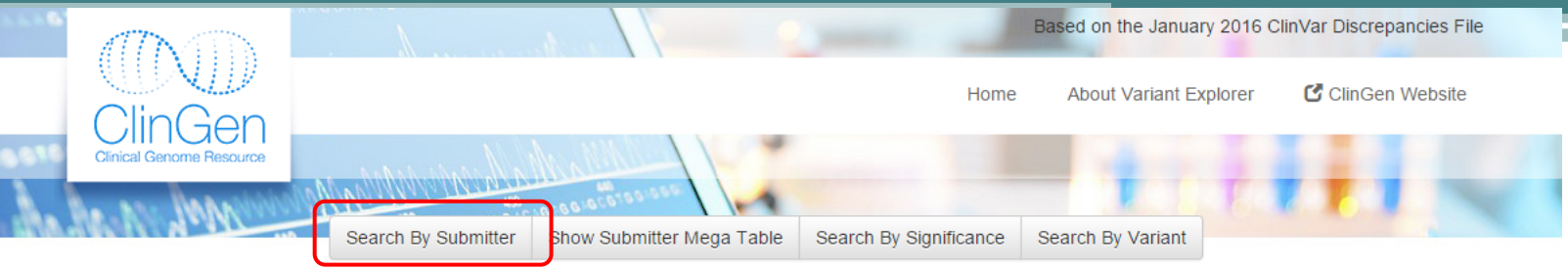
Laboratory: Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine

Lab by Lab Summary

Lab Name	Conflict	Confidence Discrepancy	Total
ARUP Laboratories University of Utah, Department of Pathology	2	1	3
Agnes Ginges Centre for Molecular Cardiology,Centenary Institute	6	3	9
Ambry Genetics	2	7	9
Baylor Miraca Genetics Laboratories	0	1	1
Biesecker Laboratory - ClinSeq Project, NHGRI	160	72	232
Blueprint Genetics	38	14	52
CSER_CC_NCGL; University of Washington Medical Center	19	5	24
Cardiovascular Biomedical Research Unit Royal Brompton & Harefield NHS Foundation Trust	30	3	33
Counsyl	0	18	18
Department of Neurology, University Hospital of Strasbourg	1	0	1
Department of Ophthalmology and Visual Sciences Kyoto University	0	5	5
Developmental Genetics Unit; King Faisal Specialist Hospital and Research Center	7	0	7
Division of Human Genetics,Children's Hospital of Philadelphia	1	1	2
Emory Genetics Laboratory	198	140	338
Evolutionary and Medical Genetics Laboratory, Centre for Cellular and Molecular Biology	2	0	2
GeneDx	350	420	770
GeneReviews	7	3	10

variantexplorer.org

Justin Aronson, Steven Harrison, Larry Babb, Sandy Aronson, Heidi Rehm



Laboratory: Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine

Lab by Lab Summary

Lab Name	Conflict	Confidence Discrepancy	Total
ARUP Laboratories University of Utah, Department of Pathology	2	1	3
Agnes Ginges Centre for Molecular Cardiology,Centenary Institute	6	3	9
Ambry Genetics			
Baylor Miraca Genetics Laboratories			
Biesecker Laboratory - ClinSeq Project, NHGRI			
Blueprint Genetics			
CSER_CC_NCGL; University of Washington Medical Center			
Cardiovascular Biomedical Research Unit Royal Brompton & Harefield NHS Foundation Trust			
Counsyl			
Department of Neurology, University Hospital of Strasbourg			
Department of Ophthalmology and Visual Sciences Kyoto University			
Developmental Genetics Unit; King Faisal Specialist Hospital and Research Center			
Division of Human Genetics,Children's Hospital of Philadelphia	1	1	2
Emory Genetics Laboratory	198	140	338
Evolutionary and Medical Genetics Laboratory, Centre for Cellular and Molecular Biology	2	0	2
GeneDx	350	420	770
GeneReviews	7	3	10

Significance Name	Pathogenic	Likely pathogenic	Uncertain significance	Likely benign	Benign
Pathogenic	0	51	4	0	0
Likely pathogenic	196	0	16	1	1
Uncertain significance	212	165	0	201	61
Likely benign	20	7	301	0	486
Benign	15	8	83	340	0

variantexplorer.org

Justin Aronson, Steven Harrison, Larry Babb, Sandy Aronson, Heidi Rehm

ClinGen Inter-Laboratory Discrepancy Resolution WG

1. Identify variants interpreted by ≥2 labs
2. Reassess variants using ACMG/AMP guidelines
3. Share internal evidence
4. Identify persistent interpretation differences due to varying application of ACMG/AMP rules
5. Assess reason for initial discordant interpretation
6. Update ClinVar

Comparison of ClinVar Submitted Variants Across Four Labs:

Ambry, GeneDx, Partners LMM, Univ. Chicago - **49,734 unique variants**

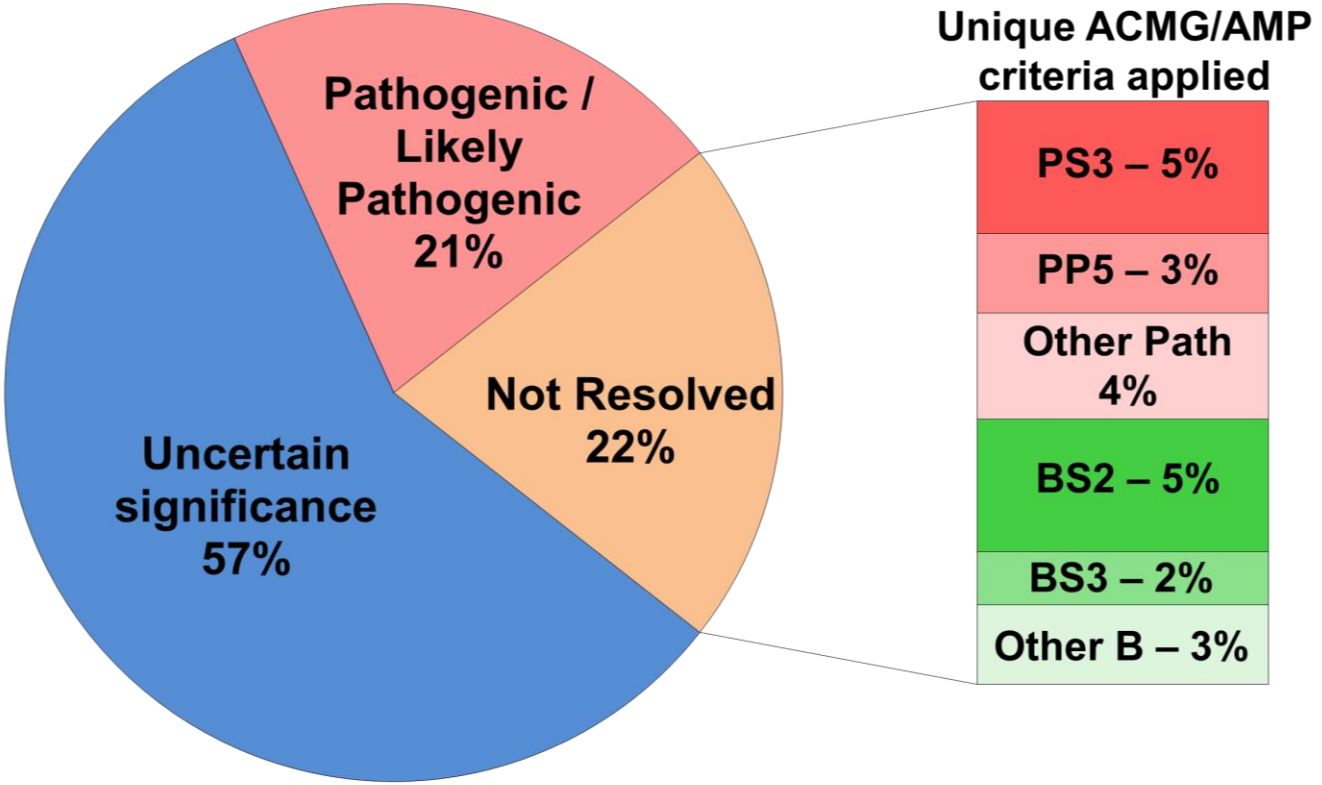
1. Identify variants interpreted by ≥2 labs

Submitted by	# shared variants	# Agreed (%)	# VUS vs. LB/B differences	# P/LP vs. VUS/LB/B differences
Lab 1 / Lab 2	2318	2035 (88%)	125 (5%)	158 (7%)
Lab 3 / Lab 1	2312	2068 (89%)	200 (9%)	44 (2%)
Lab 1 / Lab 4	1256	1086 (86%)	160 (13%)	10 (1%)
Lab 4 / Lab 2	513	478 (93%)	30 (6%)	5 (1%)
Lab 3 / Lab 4	86	77 (90%)	9 (10%)	0
Lab 3 / Lab 2	65	62 (95%)	2 (3%)	1 (2%)
All 4 Labs	6169	5445 (88%)	508 (8%)	216 (4%)

Steven Harrison, Jill Dolinsky, Lisa Vincent, Amy Knight Johnson, Danielle Azzariti, Tina Pesaran, Elizabeth Chao, Soma Das, Sherri Bale, Heidi Rehm

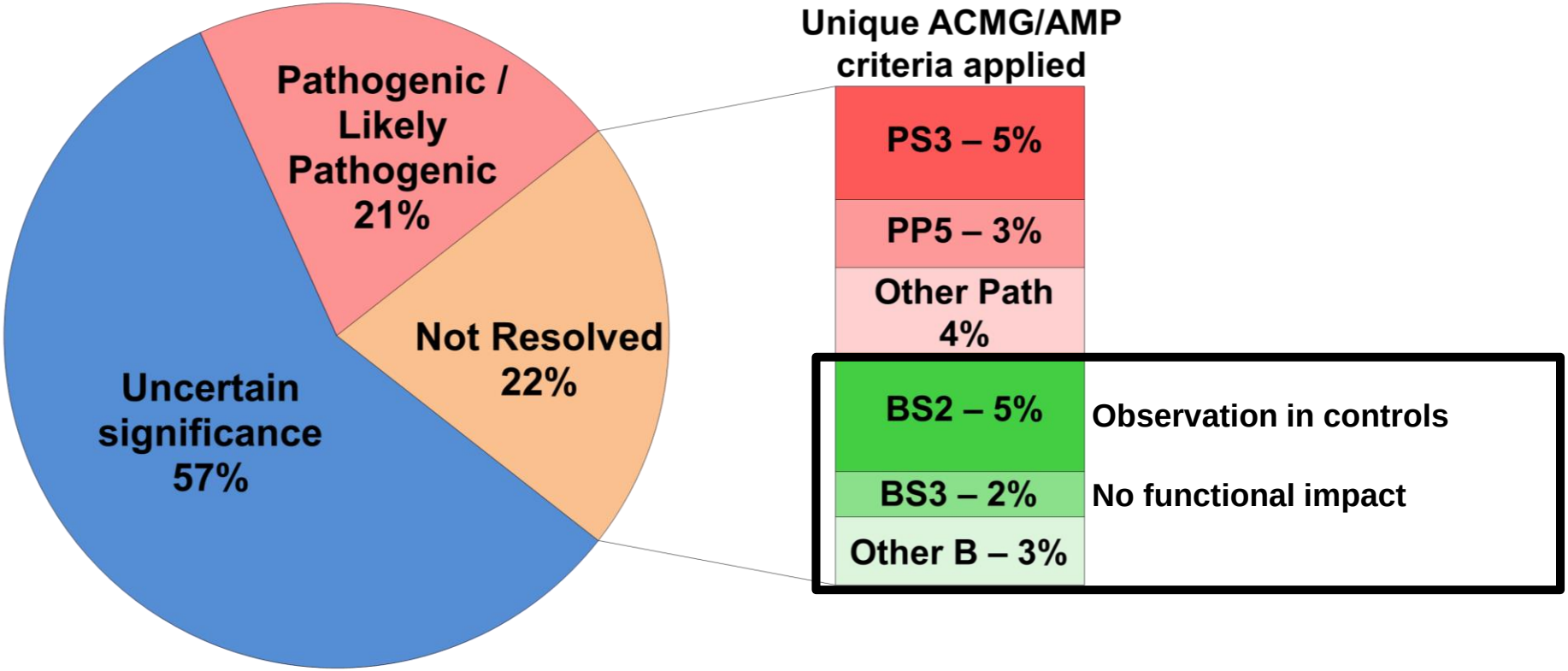
Comparison of ClinVar Submitted Variants Across Four Labs

Resolution Outcome of 104 Reassessed P/LP vs VUS/LB/B differences



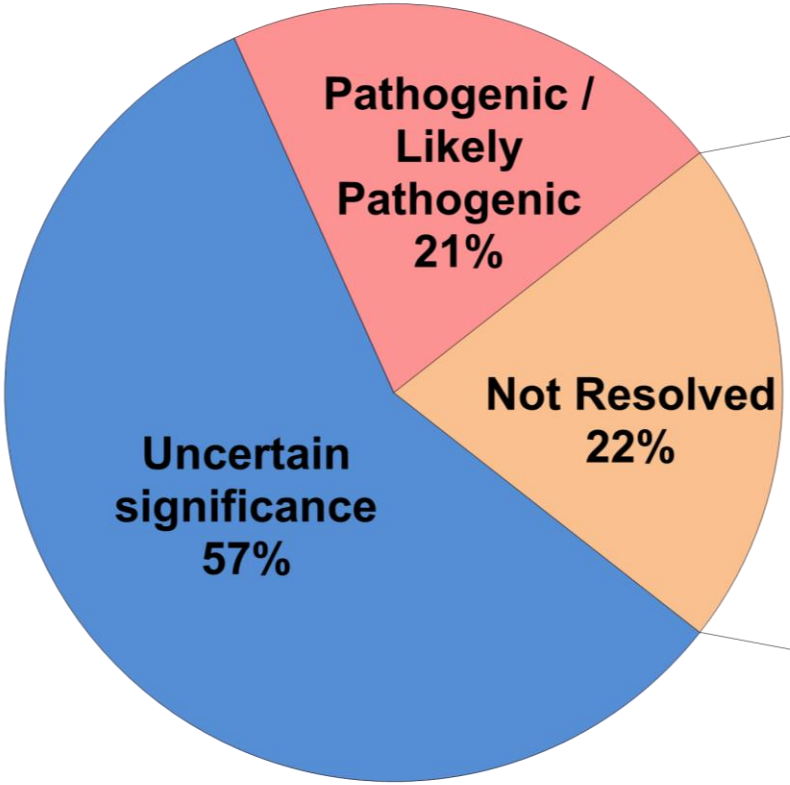
Comparison of ClinVar Submitted Variants Across Four Labs

Resolution Outcome of 104 Reassessed P/LP vs VUS/LB/B differences



Comparison of ClinVar Submitted Variants Across Four Labs

Resolution Outcome of 104 Reassessed P/LP vs VUS/LB/B differences

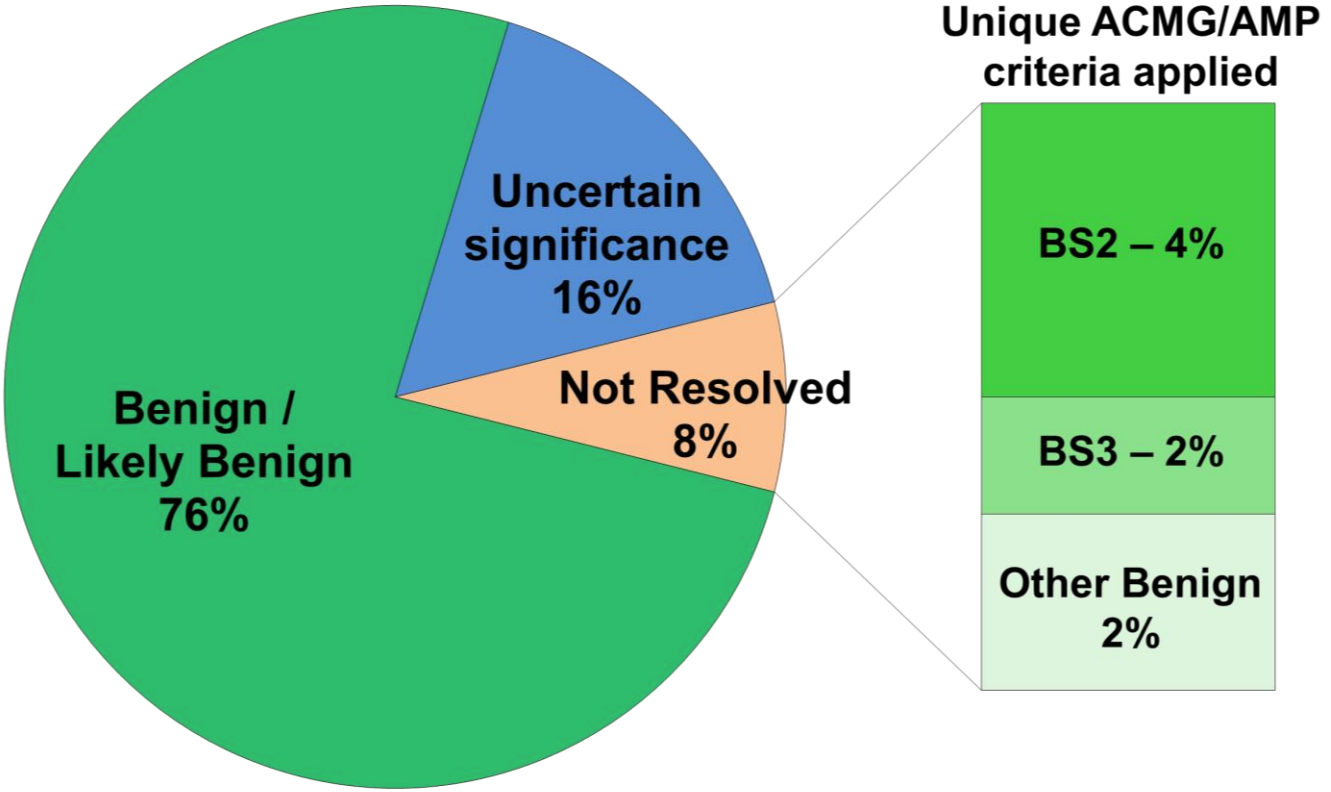


Unique ACMG/AMP criteria applied

PS3 – 5%	Functional studies
PP5 – 3%	Reputable source
Other Path 4%	Hotspot/functional domain
BS2 – 5%	Observation in controls
BS3 – 2%	No functional impact
Other B – 3%	

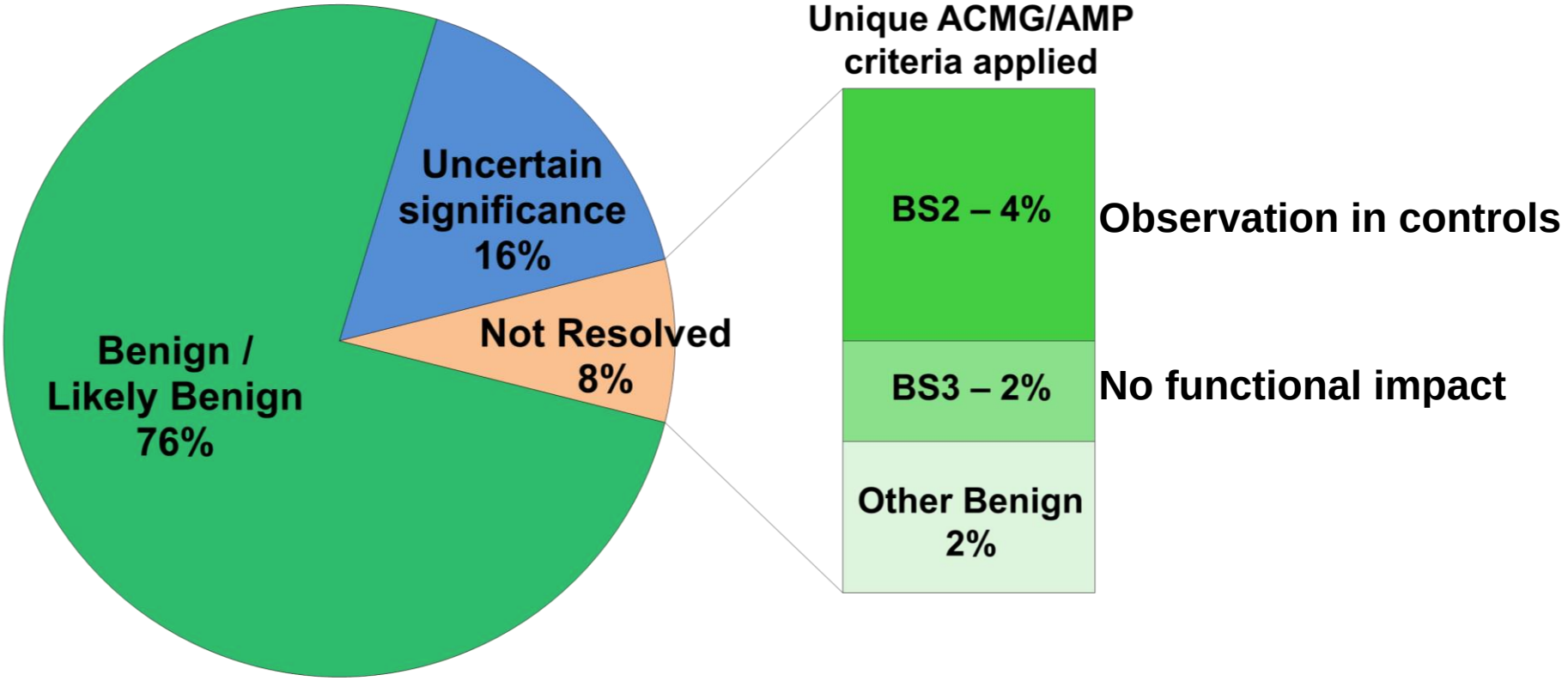
Comparison of ClinVar Submitted Variants Across Four Labs

Resolution Outcome of 128 Reassessed VUS vs. LB/B differences

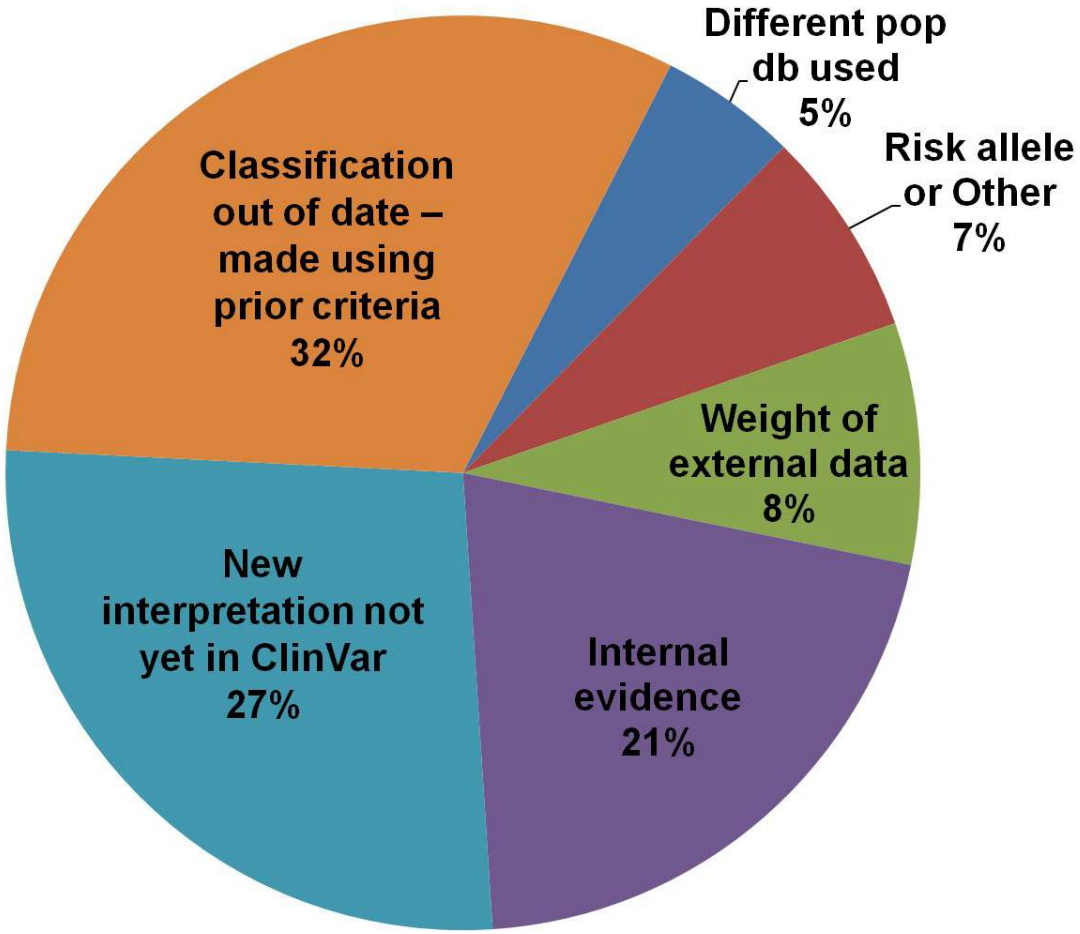


Comparison of ClinVar Submitted Variants Across Four Labs

Resolution Outcome of 128 Reassessed VUS vs. LB/B differences



Basis for Interpretation Differences for 87 resolved variants



- Out of date classifications accounted for most discrepancies
  - Lab reassess rules
- Internal evidence facilitated 21% resolutions

Comparison of ClinVar Submitted Variants Across Four Labs:

Ambry, GeneDx, Partners LMM, Univ. Chicago - **49,734 unique variants**

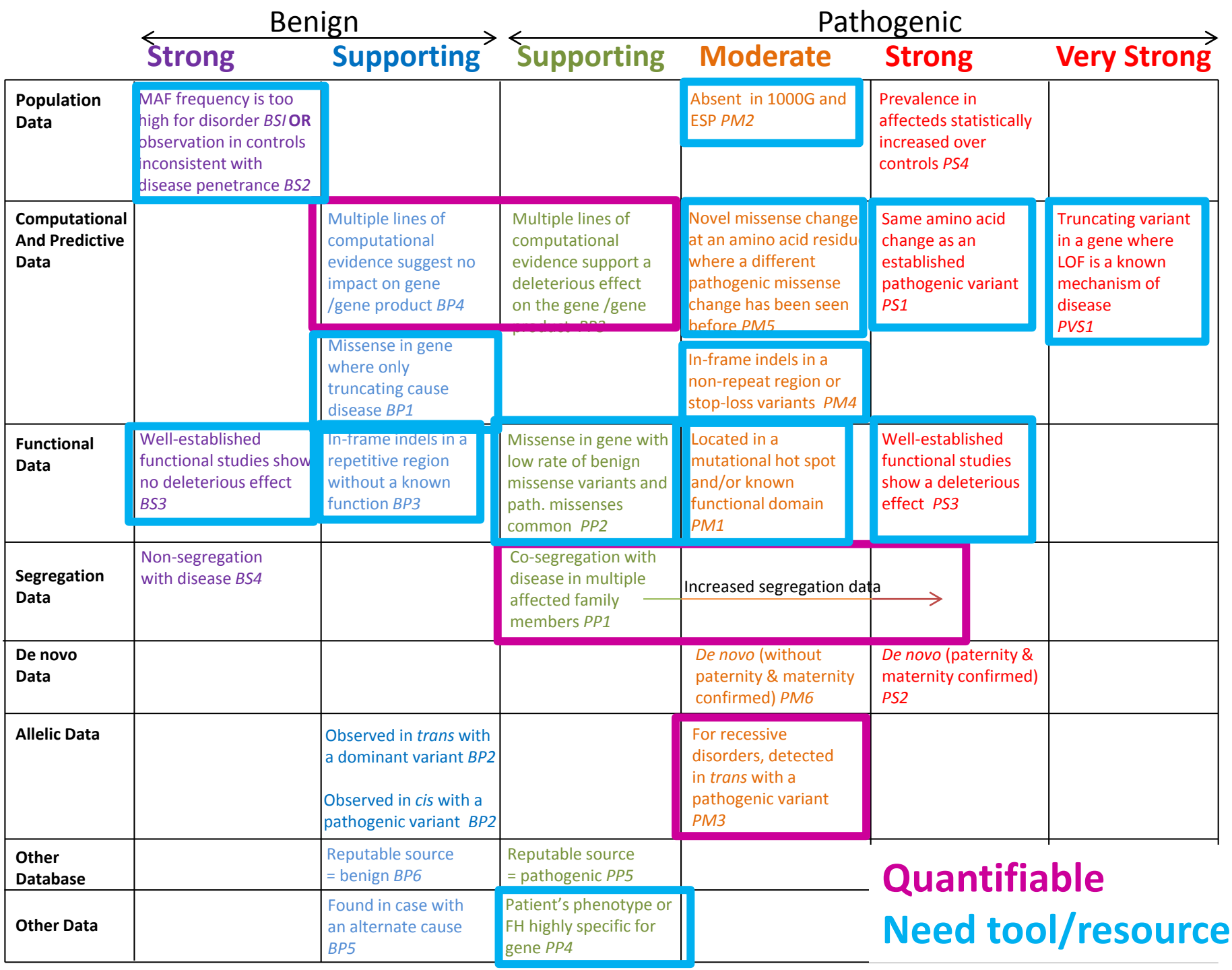
Submitted by	# shared variants	# Agreed (%)	# VUS vs. LB/B differences	# P/LP vs. VUS/LB/B differences
Lab 1 / Lab 2	2318	2035 (88%)	125 (5%)	158 (7%)
Lab 3 / Lab 1	2312	2068 (89%)	200 (9%)	44 (2%)
Lab 1 / Lab 4	1256	1086 (86%)	160 (13%)	10 (1%)
Lab 4 / Lab 2	513	478 (93%)	30 (6%)	5 (1%)
Lab 3 / Lab 4	86	77 (90%)	9 (10%)	0
Lab 3 / Lab 2	65	62 (95%)	2 (3%)	1 (2%)
All 4 Labs	6169	5445 (88%)	508 (8%)	216 (4%)
		5645 (92%)	398 (6%)	126 (2%)

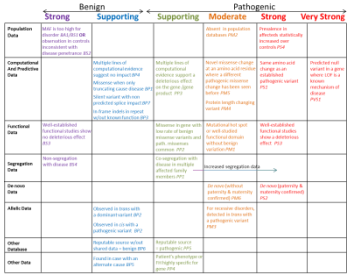
86% (200/232) resolved

Steven Harrison, Jill Dolinsky, Lisa Vincent, Amy Knight Johnson, Danielle Azzariti, Tina Pesaran, Elizabeth Chao, Soma Das, Sherri Bale, Heidi Rehm

Lessons Learned

- The majority of differences in variant classification are resolvable through consensus and data sharing
- Variant classification often requires professional judgment (even when using the same rules) and therefore complete consensus may not occur
- But all evidence must be accessible and rules should be applied correctly
- The ACMG/AMP rules would benefit from added quantitative guidance as well as gene/disease specific guidance


Benign
Pathogenic
Strong
Supporting
Supporting
Moderate
Strong
Very Strong
Population Data
MAF frequency is too high for disorder BS1 OR observation in controls inconsistent with disease penetrance BS2
Absent in 1000G and ESP PM2
Prevalence in affecteds statistically increased over controls PS4
Computational And Predictive Data
Multiple lines of computational evidence suggest no impact on gene /gene product BP4
Multiple lines of computational evidence support a deleterious effect on the gene /gene
Novel missense change at an amino acid residu where a different pathogenic missense change has been seen before PM5
Same amino acid change as an established pathogenic variant PS1
Truncating variant in a gene where LOF is a known mechanism of disease PVS1
Missense in gene where only truncating cause disease BP1
In-frame indels in a non-repeat region or stop-loss variants PM4
Functional Data
Well-established functional studies show no deleterious effect BS3
In-frame indels in a repetitive region without a known function BP3
Missense in gene with low rate of benign missense variants and path. missenses common PP2
Located in a mutational hot spot and/or known functional domain PM1
Well-established functional studies show a deleterious effect PS3
Segregation Data
Non-segregation with disease BS4
Co-segregation with disease in multiple affected family members PP1
Increased segregation data
De novo Data
De novo (without paternity & maternity confirmed) PM6
De novo (paternity & maternity confirmed) PS2
Allelic Data
Observed in trans with a dominant variant BP2
Observed in cis with a pathogenic variant BP2
For recessive disorders, detected in trans with a pathogenic variant PM3
Other Database
Reputable source = benign BP6
Reputable source = pathogenic PP5
Other Data
Found in case with an alternate cause BP5
Patient's phenotype or FH highly specific for gene PP4
Quantifiable
Need tool/resource



ACMG/AMP Rules

Interlab Seq Var Discrepancy Resolution Task Team

- Cardiomyopathy Expert Panel
- Noonan Spectrum Expert Panel
- Hereditary Cancer Expert Panels
- Metabolic Disease Expert Panel
- Developmental Delay Expert Panel
- Others…….

Gene and disease-specific ACMG/AMP rule specification (frequency thresholds, acceptable functional assays, etc)

ClinGen Sequence Variant Interpretation Work Group
(Co-Chairs Les Beisecker and Marc Greenblat)

Short term: Refine and clarify current ACMG/AMP criteria
Medium term: Modify ACMG/AMP criteria
Long term: Move to quantitative Bayesian framework

Optimization and Utilization of ACMG Variant Classification Criteria for the RASopathies: A ClinGen Initiative

Lisa M. Vincent ,Heather Mason-Suares, Rong Mao, Mitchell W. Dillon, Brad Williams, Patroula Smpokou, Karen W. Gripp, Katherine A. Rauen, Amy E. Roberts, Bruce D. Gelb, and Sherri Bale

Table 1: Assessment of Strength of Evidence Relative to RASopathy Spectrum

		Evidence Requirements			
PATHOGENIC CRITERIA	**OFFICIAL ACMG CRITERIA** [Richards et al. 2015]	**VERY STRONG**	**STRONG**	**MODERATE**	**SUPPORTING**
PS2	De novo (both maternity and paternity confirmed) in a patient with the disease and no family history	≥2 independent occurrences (PVS_NP9)			
PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product		≥2 unique in vitro or in vivo functional studies OR ≥2 independent groups with concordant deleterious results for the same assay *if no formal assays approved by expert panel available (PS_NP2)*	One in vitro or in vivo functional studies *if no formal assays approved by expert panel available (PM_NP8)*	
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before		≥2 different pathogenic missense changes (PS_NP3)		
PM6	Assumed de novo, but without confirmation of paternity and maternity	≥2 independent occurrences plus 1 occurrence of PS2 (PVS_NP9)	≥2 independent occurrences (PS_NP1)		
PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease		≥7 meioses (PS_NP4)	≥5 meioses (PM_NP6)	≥2 meioses
BENIGN CRITERIA	**OFFICIAL ACMG CRITERIA** [Richards et al. 2015]	**STAND-ALONE**	**STRONG**	**SUPPORTING**	
BA1 BS1	Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC Allele frequency is greater than expected for disorder	An allele frequency ≥0.05% subject to a 95% confidence interval based on the population size and a minimum of 5 alleles present in the population. (BA_NB1)	An allele frequency ≥0.025% subject to a 95% confidence interval based on the population size and a minimum of 5 alleles present in the population. Based on disease prevalence of 1:1000		
BS3	Well-established in vitro or in vivo functional studies shows no damaging effect on protein function or splicing		≥2 unique in vitro or in vivo functional studies OR ≥2 independent groups with concordant benign results for the same assay *if no formal assays approved by expert panel available*	One in vitro or in vivo functional studies *if no formal assays approved by expert panel available (BP_NB5)*	
		≥2 meioses (BA_NB2)	≥1 meiosis		
			≥2 independent occurrences where increased clinical severity of disease is not evident (BS_NB4)		
			≥2 independent occurrences where increased clinical severity of disease is not evident (BS_NB4)		

Table 2: Other RASopathy Specific Assessments

	OFFICIAL ACMG CRITERIA [Richards et al. 2015]	ASSESSMENTS
PS1 PM1 PM5	Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation / Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Can also be applied for the same analogous residue positions/regions in highly analogous groupings: Group 1: HRAS, NRAS, KRAS Group 2: MAP2K1, MAP2K2
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC	The variant must be completely absent from all population databases. Retrospective analysis of the most common pathogenic variant in each of these genes indicated that at most only 1 allele was observed in these large control population databases suggesting the variants should be completely absent unless the variant is well-established as pathogenic.
BS2	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age	Due to variable expressivity and severity, extensive clinical workup for RASopathy spectrum features is warranted, thus general population data should not be used for this criterion unless there are observed homozygous individuals.
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	Also applicable for intronic or non-coding variants and also can be used in conjunction with BP4.

Table 3: Additional RASopathy- Specific Criteria

BENIGN- SUPPORTING	Truncating variant (nonsense, frameshift, affects canonical splice sites, initiation codon, entire gene or multi exon deletion) when disease mechanism is gain-of-function and dosage sensitivity information is consistent (BP_NB6)
BENIGN- SUPPORTING	Located in a region/domain of the protein that tolerates variation and lacks pathogenic variants (BP_NB7)

RASopathy (Noonan spectrum)

12 genes: BRAF, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1, and SOS2

12 genes: BRAF, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1, and SOS2

- Add specificity to ACMG/AMP guidelines with:
  - Gene-specific data, such as:
    - Functional domains / hot spots
    - Validated functional assays
  - Disease specific data, such:
    - Prevalence & penetrance
    - Disease mechanisms

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data	***Defined:*** ***BA1 – 0.05%*** ***BS1 – 0.025%***	Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Defined:
BA1 – 0.05%
BS1 – 0.025%

Very Strong
≥ 2 cases

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1			
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Defined:
BA1 – 0.05%
BS1 – 0.025%

Moderate
Non-approved assay

Defined assays

Increased segregation data

Very Strong
≥ 2 cases

RASopathy (Noonan spectrum)

Assess reliability of refined criteria using ~15 variants in each gene with different classifications

- ~5 variants deemed historically pathogenic by literature review
- ~5 variants with ***consistent*** ClinVar classifications (≥2 submitters)
- ~5 variants with ***different*** ClinVar classifications (≥2 submitters)

- Expert panel concordance on 82/83 variants reassessed with RASopathy-customized ACMG guidelines
- RASopathy-customized ACMG criteria influenced
  - ~58% (n=48) with criteria strength adjustments
  - ~47% (n=39) with criteria based on curated gene-specific data
  - ~8% (n=7) with new RASopathy specific criteria

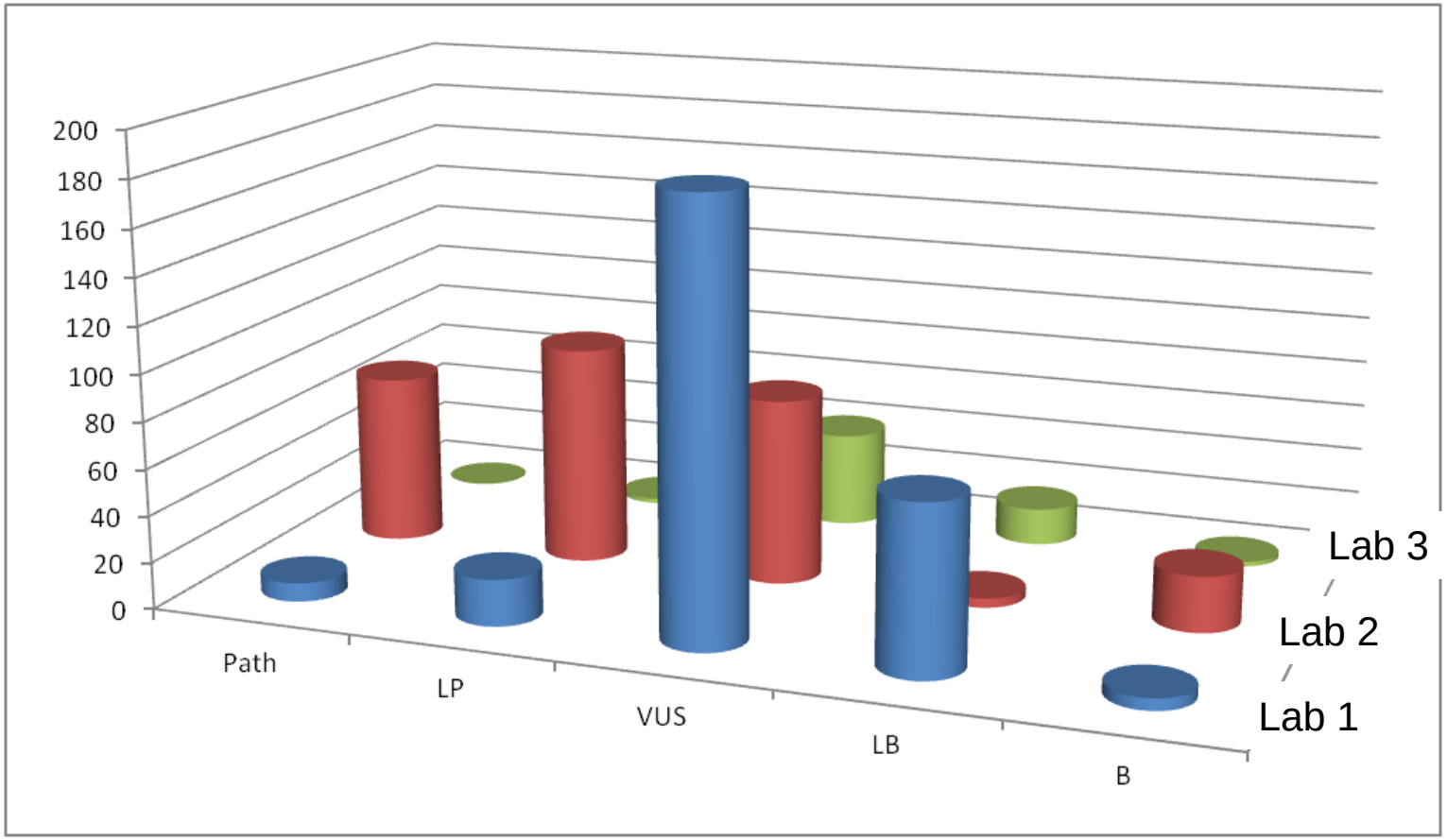
- Sharing clinical laboratory data influenced ~26% (12/46) classification calls with insufficient historic or literature-based data

Cardiomyopathy – MYH7

Develop framework + process for establishing validity of

variant-disease relationships

***MYH7* variant classifications across 3 ClinVar submitters**

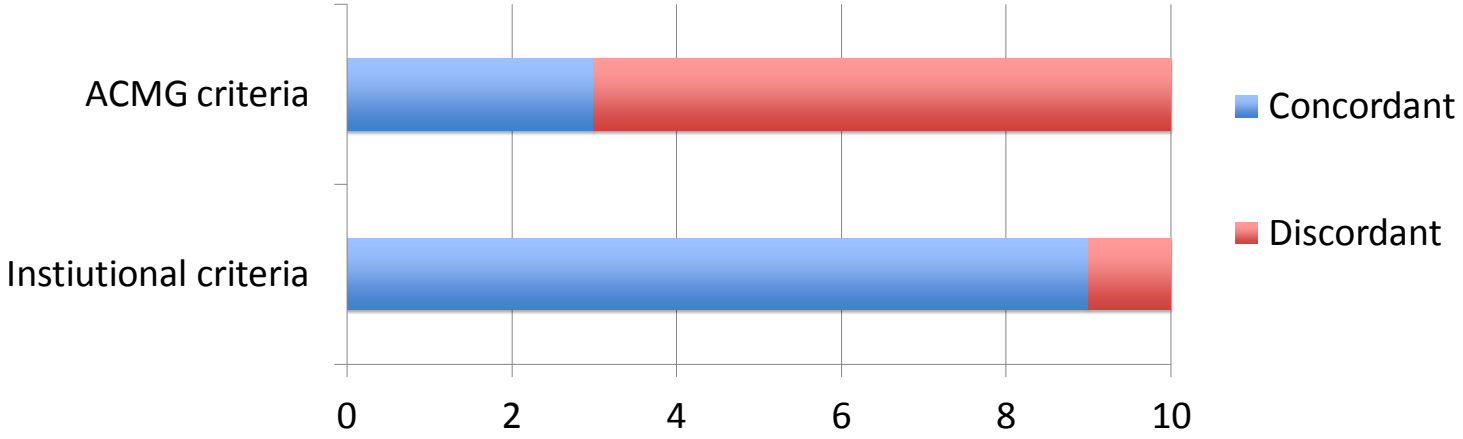


Birgit Funke

3 cardiomyopathy experts classified 10 *MYH7* variants 2 x

1) Institutional rules

2) ACMG rules



- Lack of familiarity with ACMG rules
- Lack of ACMG rule specificity

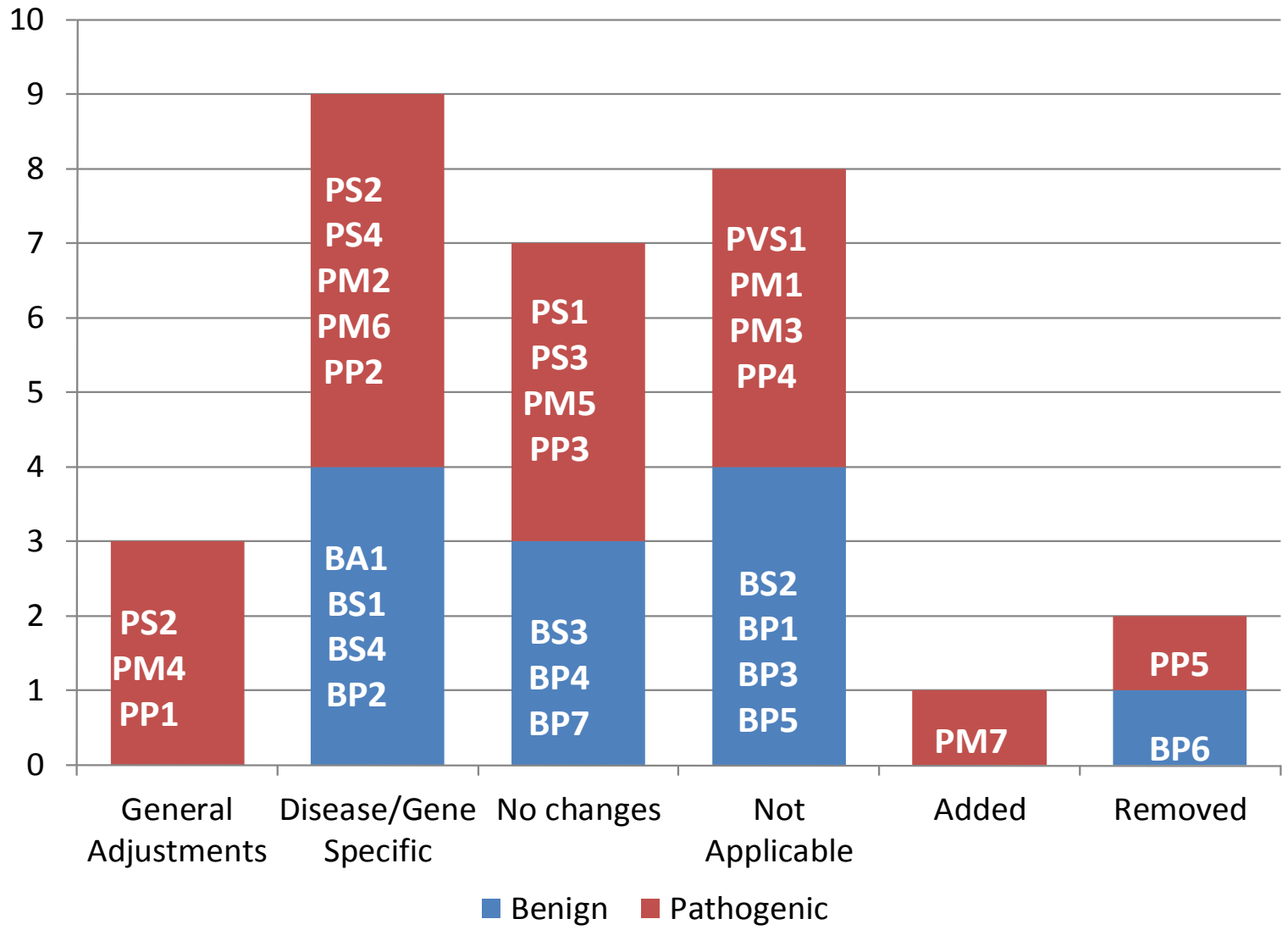
Birgit Funke

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

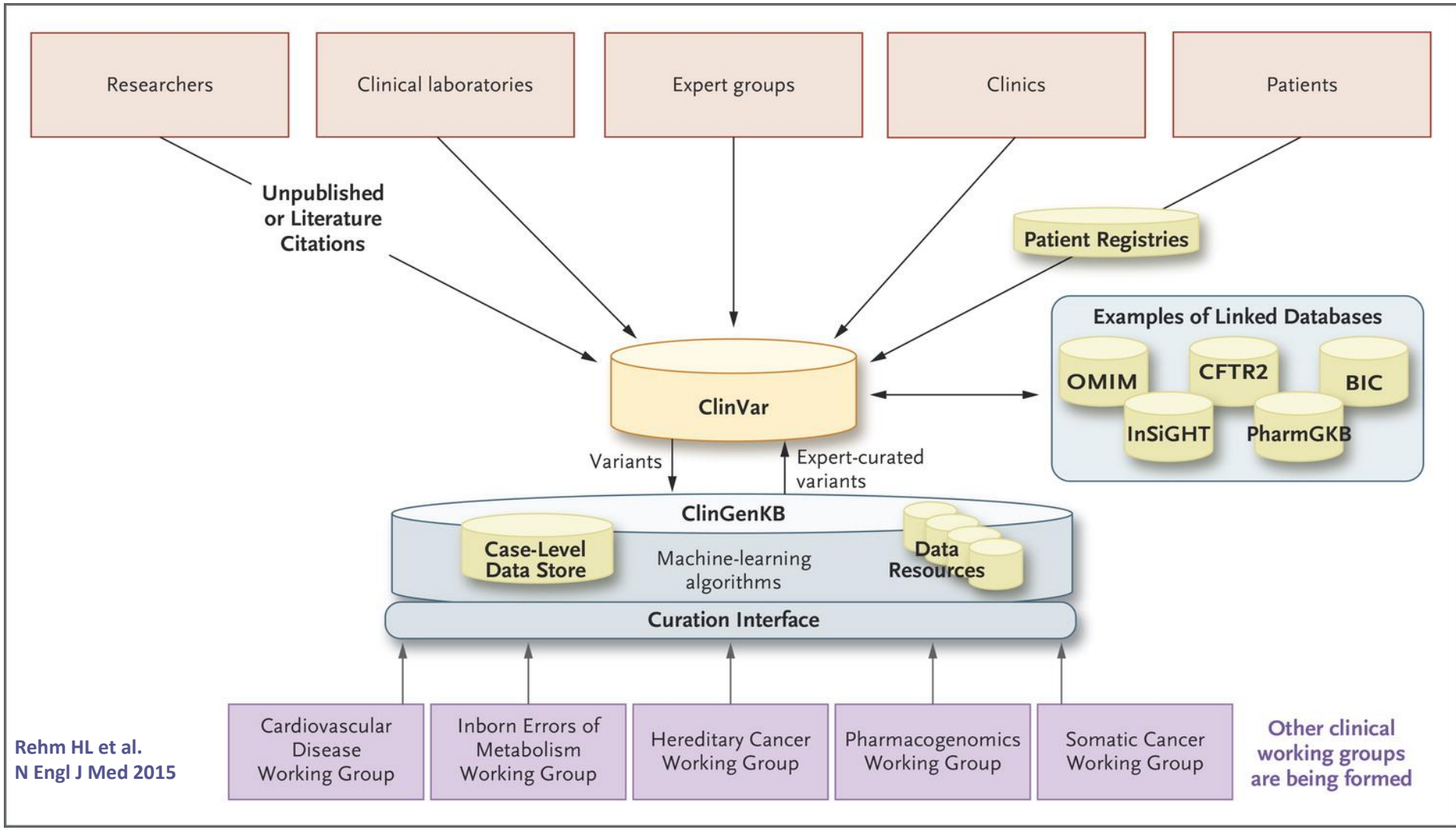
	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source found to share data on variant BP6	Reputable source found to share data on variant PP4			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very strong**
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1 *3 segs*	Increased segregation data → *5 segs*	*7 segs*	
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source without shared data on variant BP6	Reputable source with shared data on variant = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Summary MYH7 ACMG Guideline Specification

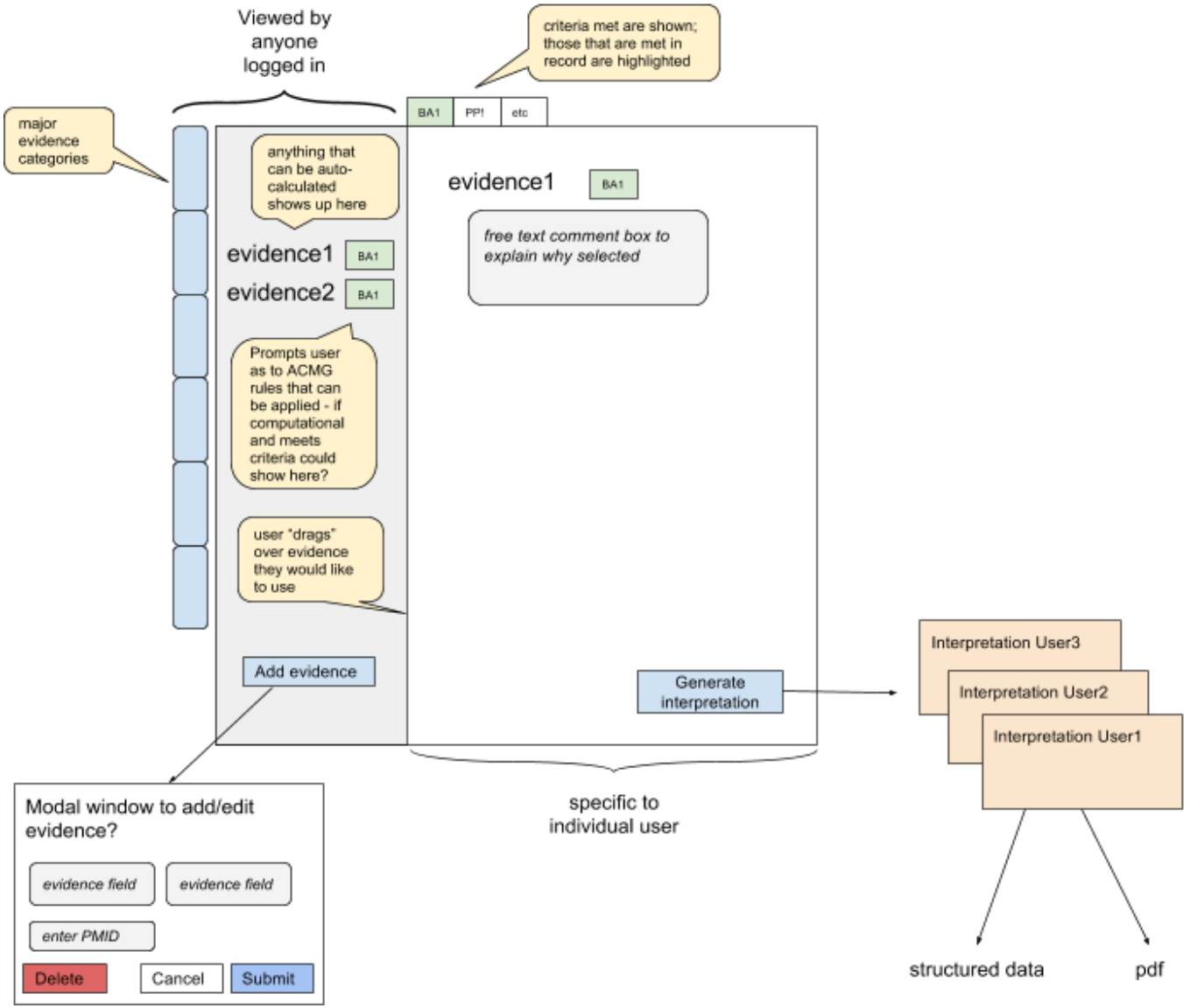


Birgit Funke



Rehm HL et al.
N Engl J Med 2015

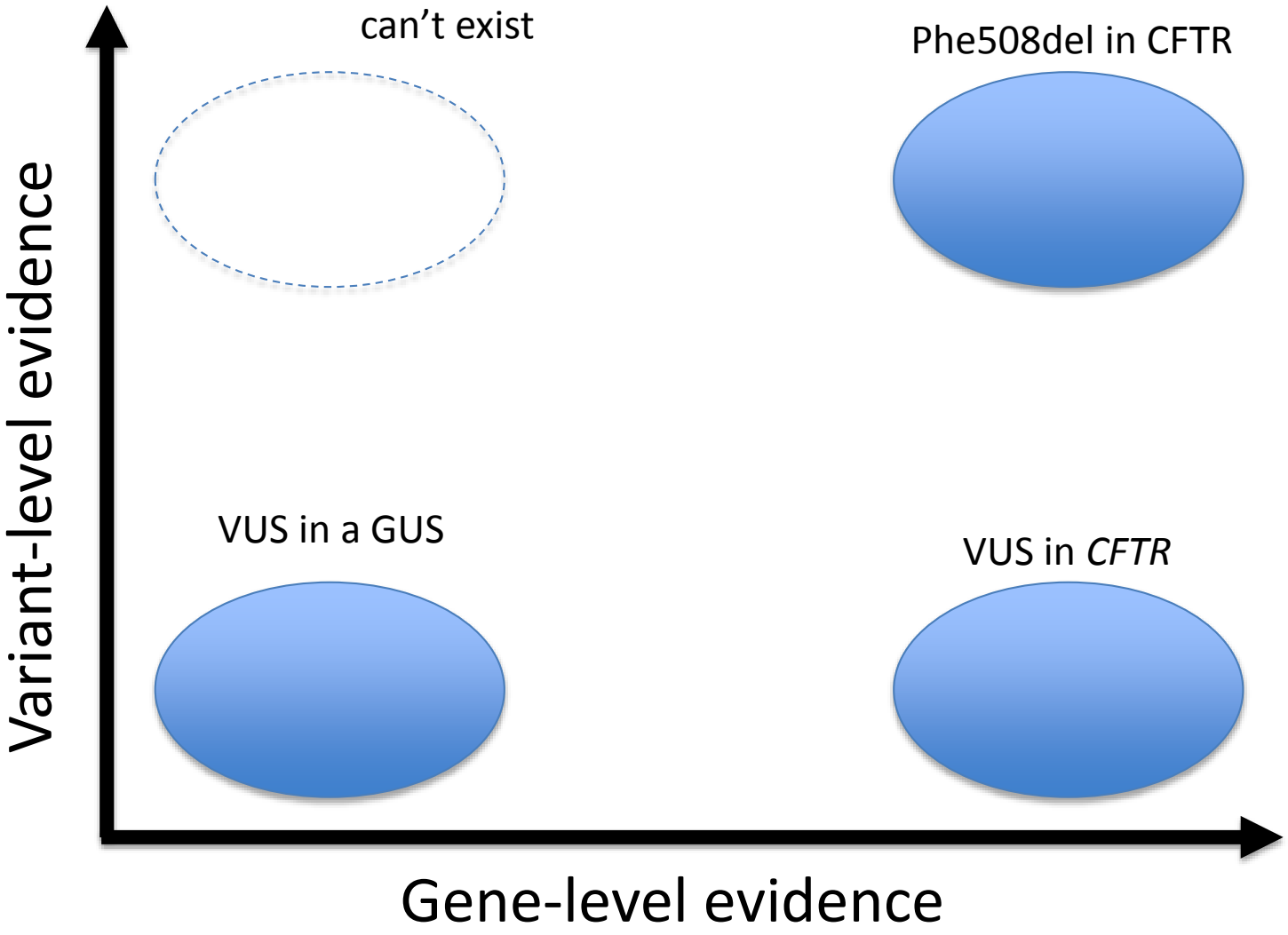
ClinGen Variant Curation Interface (in progress)



What about genes?

How do we evaluate whether a gene has sufficient evidence for an association with disease?

The two axes of implication



Modified from Daniel MacArthur

ClinGen Gene-Disease Validity Classification

Definitive	Role has been repeatedly demonstrated in research & clinical diagnostic settings • Upheld over time (in general, at least 3 years) • No convincing contradictory evidence
Strong	≥2 independent studies with: • Multiple pathogenic variants in unrelated probands • AND • Several different types of supporting experimental data • OR • Excess of pathogenic variants in cases vs. controls • No convincing contradictory evidence
Moderate	≥1 independent study with: • ≥3 unrelated probands with pathogenic variants • Some supporting experimental data • No convincing contradictory evidence
Limited	≥1 independent study with: • <3 unrelated probands with pathogenic variants • OR • Multiple variants reported in unrelated probands but *without* sufficient evidence for pathogenicity • No convincing contradictory evidence
No Evidence Reported	No evidence reported for a causal role in disease (candidate genes, etc.), therefore no pathogenic variants have been identified in humans to date.
Conflicting Evidence Reported	
Disputed	Convincing evidence disputing a role for this gene in this disease has arisen • Disputing evidence need not outweigh existing evidence supporting the gene:disease association
Refuted	Evidence refuting the gene in the specified disease has been reported and significantly outweighs any evidence supporting the role • Applied at the discretion of clinical domain experts after thorough review of available evidence

http://www.clinicalgenome.org/knowledge-curation/gene-curation/

ClinGen Clinical Validity Summary Matrix

Assertion criteria	Description	Number of Points							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
# Probands and/or Case-Control Data	Total # of *unrelated* probands with variants that provide convincing evidence for disease causality across all curated literature or case-control data	N/A	1-3	4-6	7-9	10-12	13-15	16-18	19+
Experimental evidence	Points given based on the **gene-level** functional evidence supporting a role for this gene in disease	0	1	2	3	4	5	6+	
# Publications	# of curated Independent publications reporting **human** variants in the gene under consideration	N/A	1	2	3	4	5+		
Time (yrs)	# of years since first publication reporting a disease association **(if ≤2 publications --> then 1 is max score for time)**	this yr	1-3 yr	≥3 yr					

Is there valid contradictory evidence?	Y/N?	**Classification**	**Total Score**	**Assertion:**
Description of Contradictory Evidence:		**Limited:**	**0-8**	
		Moderate:	**9-12**	
		Strong:	**13-16**	
		Definitive:	**17-20**	

Data sharing and **expert interpretation**
will improve
our **knowledge** of DNA variation
and develop **consistency in variant classification**

Acknowledgements

ClinGen
Heidi Rehm
Christa Martin
Danielle Azzariti
Erin Riggs
Larry Babb
Selina Dwight
Matt Wright

Discrepancy
Jill Dolinsky (Ambry)
Tina Pesaran (Ambry)
Elizabeth Chao (Ambry)
Lisa Vincent (GeneDx)
Sherri Bale (GeneDx)
Amy Knight Johnson (U Chicago)
Soma Das (U Chicago)

ClinVar
Donna Maglott
Melissa Landrum
George Riley

NHGRI
Erin Ramos
Annie Niehaus

LMM
Birgit Funke
Melissa Kelly
Jillian Buchan