Program Schedule

- **Introduction to ClinGen** (Jonathan Berg, 10 mins)
- **Clinical Gene Validity** (Erin Riggs, 25 mins)
- **Variant Classification** (Heidi Rehm, 35 mins)
- **Pathogenicity Calculator** (Aleks Milosavljevic, 20 mins)

Interactive Invited Workshop: Growing the Public Knowledge Base for Clinical Genome Interpretation - Harnessing the Resources of the ClinGen Project

Workshop materials:

http://calculator.clinicalgenome.org/ashg-2015

Using the Audience Response System

From browser, respond at **PollEv.com/ashgbcm** . Click on your response and you will see "Vote recorded".

From cell phone, text "ASHGBCM" to 22333.

You will see the reply "You've joined Elke Eastaugh's session (ASHGBCM)". **You may submit your answer now.**

You will be allowed to answer each question only once.

When you're done answering all questions, text "LEAVE".

Introduction to ClinGen: Tools and Resources

Jonathan S. Berg, MD, Ph.D.
Department of Genetics, UNC School of Medicine

ASHG Workshop
October 6, 2015

Improving our knowledge of genomic variation requires a massive effort in data sharing and collaborative curation

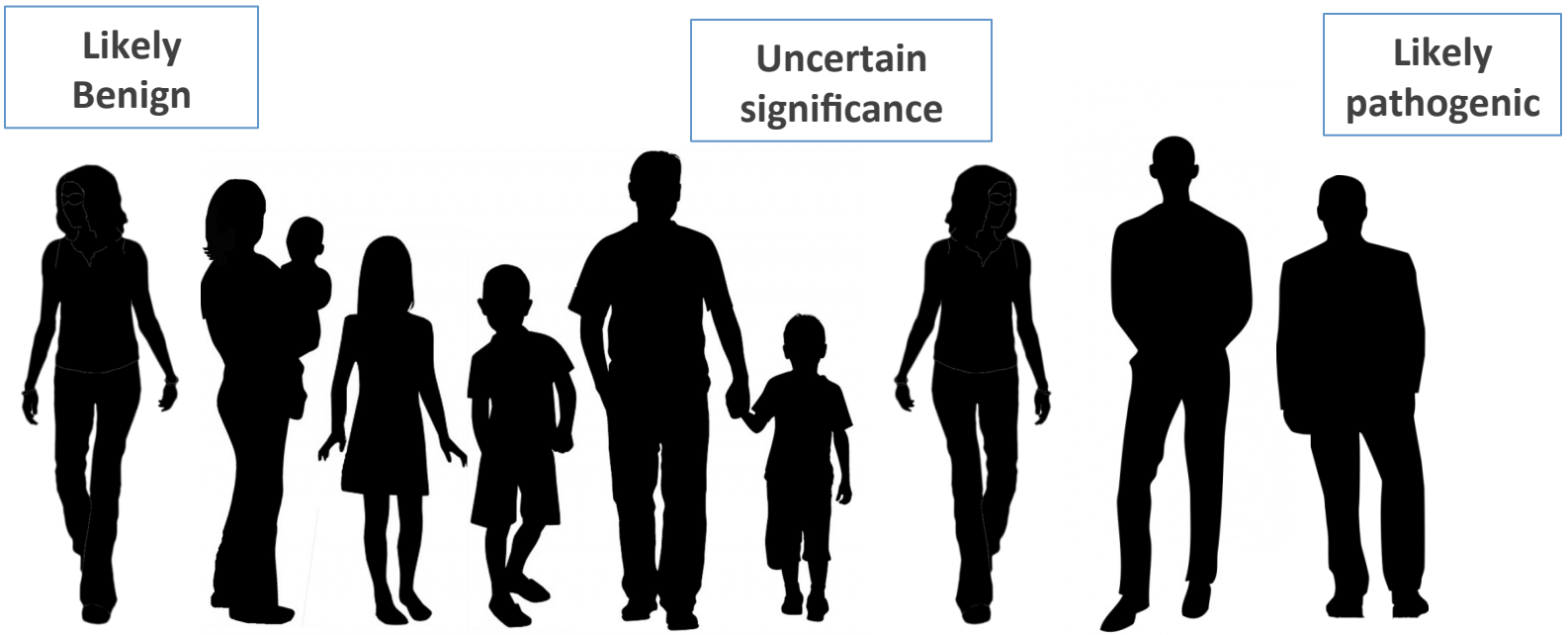
The Problem

- **>80 million genomic variants and >19,000 genes**
  - Most we don't understand

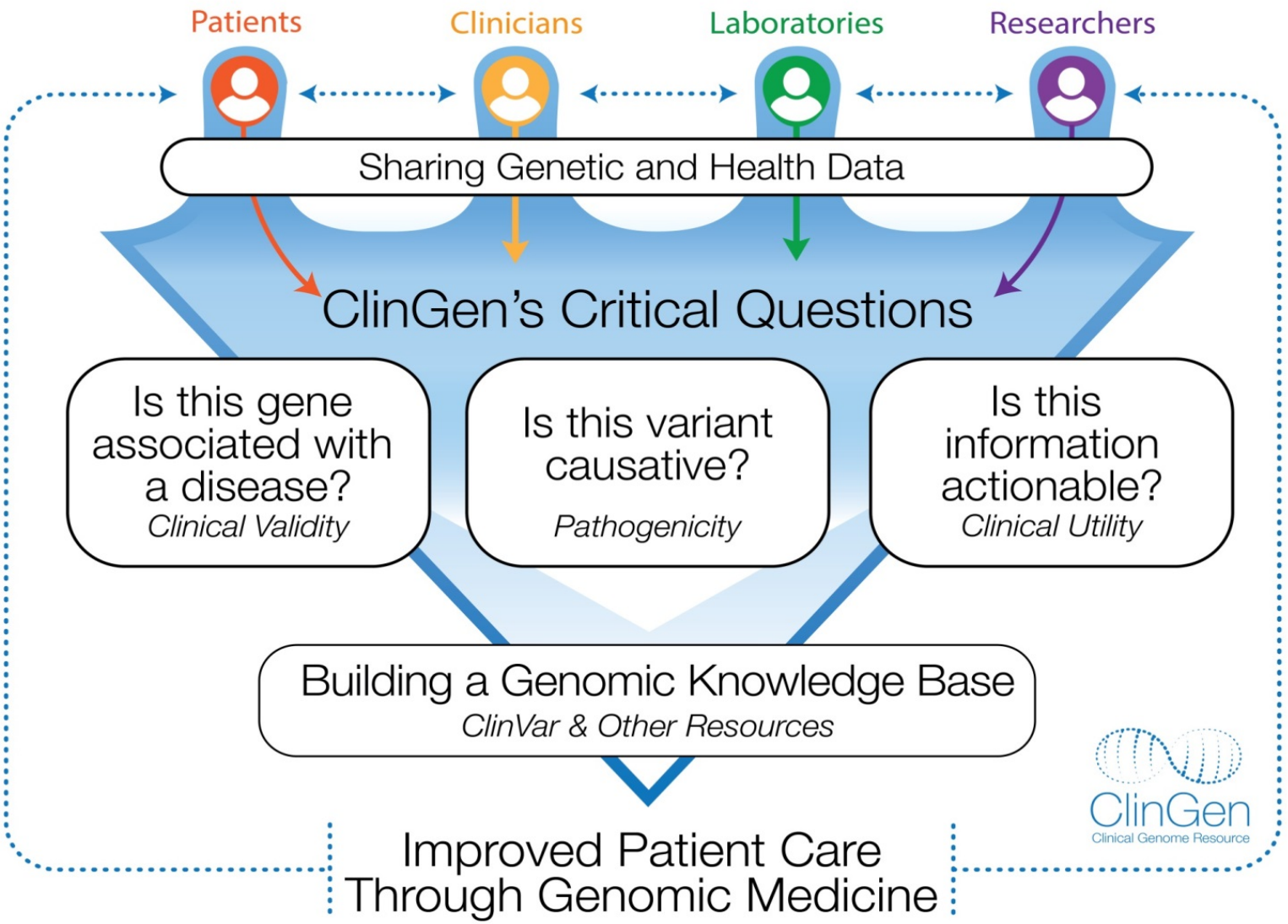
- **Ability to detect DNA variants has greatly surpassed the ability to interpret their clinical impact**

- **No centralized database or standard approaches for cataloguing this genetic data**

- In addition to the lack of a centralized database for cataloguing genetic data, **genetic testing interpretations can differ**



"Building a genomic knowledge base to improve patient care."



ClinGen Overview

https://www.clinicalgenome.org/

- **The Clinical Genome Resource (ClinGen)** aims to create an authoritative central resource that defines the clinical relevance of genomic variants for use in precision medicine and research.

- **NHGRI-funded program launched Sept. 2013**
  - FY13-FY16 = $28M Total Costs
  - 3 U grants, working closely with NCBI's ClinVar
  - Co-funding from the NICHD and NCI
  - > 375 researchers & clinicians from >90 institutions

ClinGen
Clinical Genome Resource

ClinGen Organization

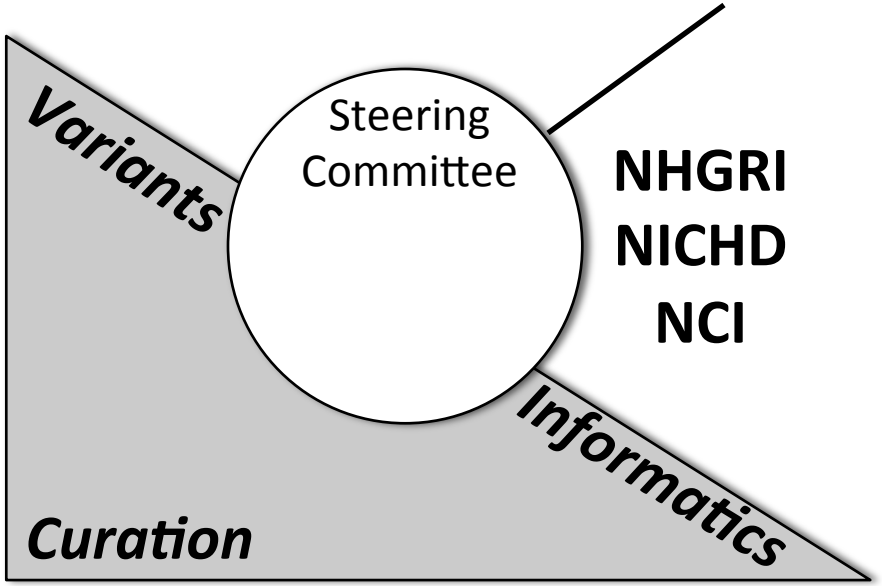
http://www.ncbi.nlm.nih.gov/clinvar/

PARTNERS HEALTHCARE

GEISINGER HEALTH SYSTEM

U41 ---------- ClinVar

National Center for Biotechnology Information NCBI



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

U01

ACMG American College of Medical Genetics and Genomics
Translating Genes Into Health®

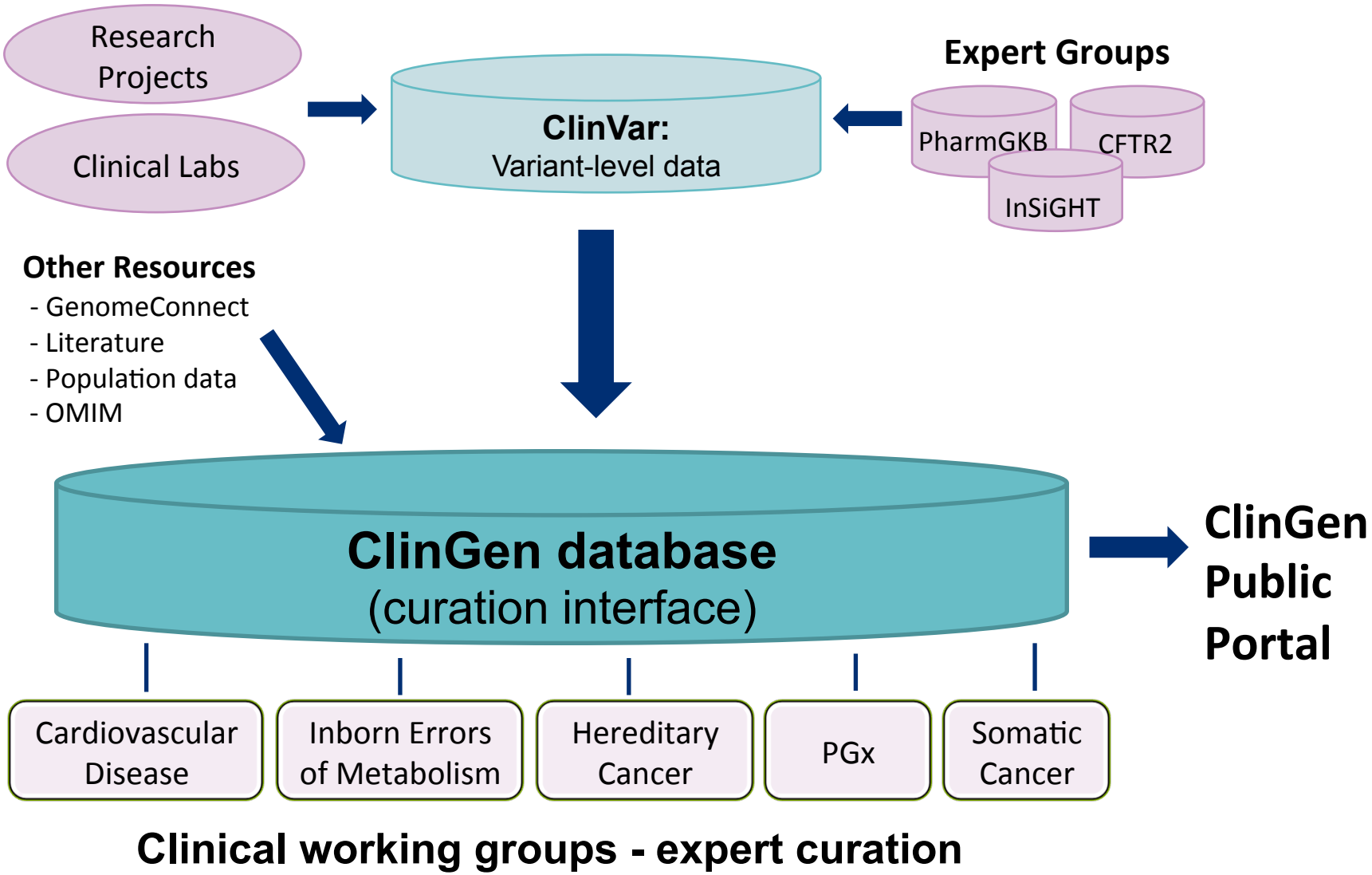
GEISINGER HEALTH SYSTEM

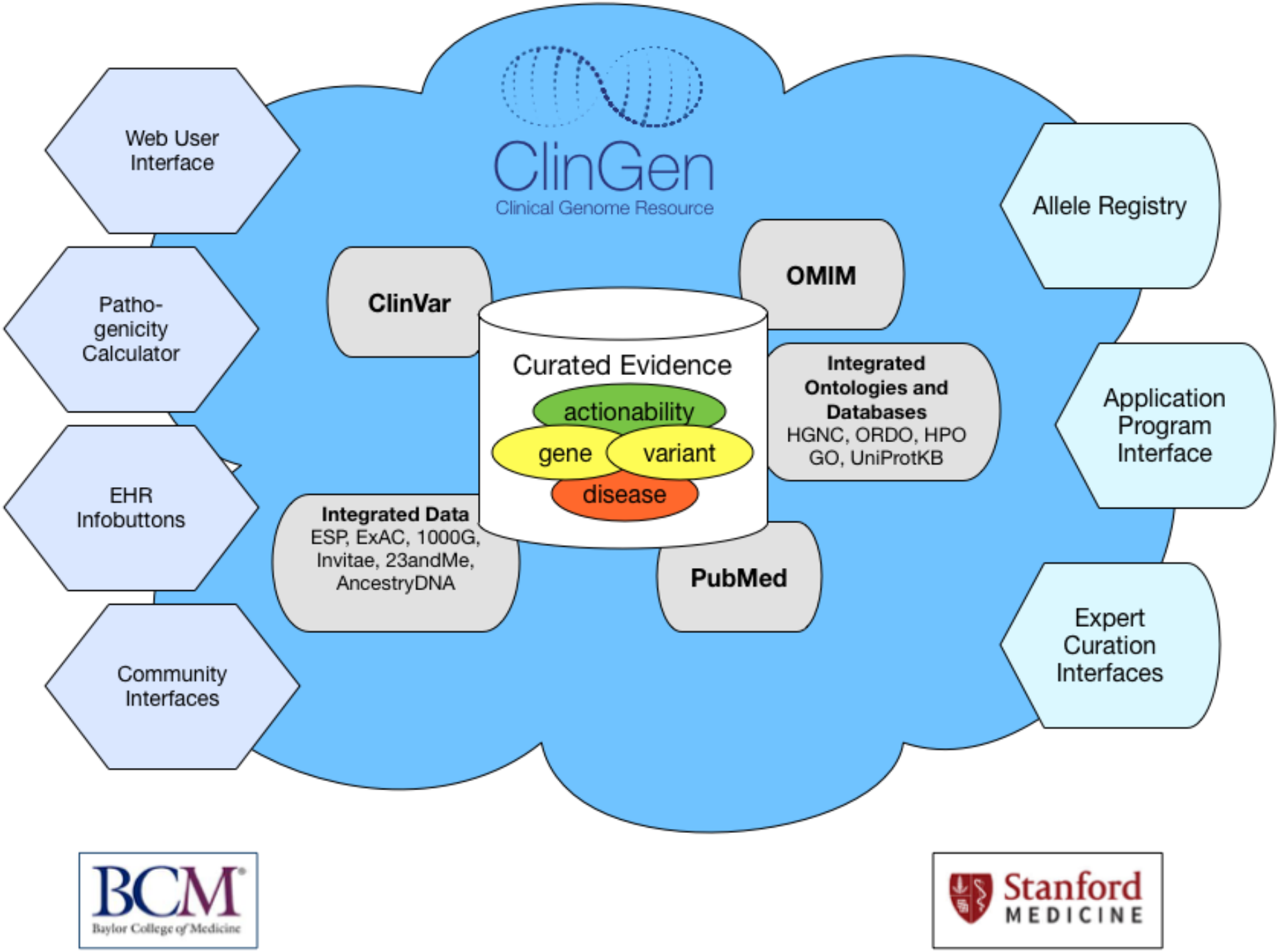
Stanford MEDICINE

U01

BCM Baylor College of Medicine

ClinGen Data Flow

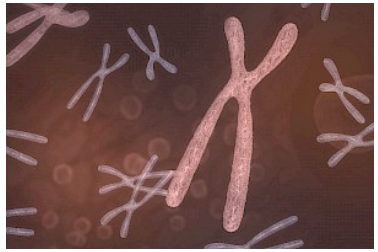



ClinGen
Clinical Genome Resource
Web User Interface
Patho-genicity Calculator
EHR Infobuttons
Community Interfaces
ClinVar
OMIM
Curated Evidence
actionability
gene
variant
disease
Integrated Ontologies and Databases
HGNC, ORDO, HPO
GO, UniProtKB
Integrated Data
ESP, ExAC, 1000G, Invitae, 23andMe, AncestryDNA
PubMed
Allele Registry
Application Program Interface
Expert Curation Interfaces
BCM
Baylor College of Medicine
Stanford
MEDICINE

ClinGen Tools and Resources

https://www.clinicalgenome.org/tools-resources/

- Dosage sensitivity map
- Array analysis toolkit
- Structural variant database (ISCA)

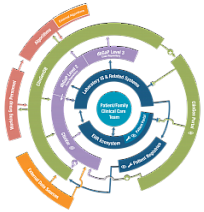


https://www.clinicalgenome.org/knowledge-curation/structural-variant-curation/

- Web resources landing page

https://www.clinicalgenome.org/tools/web-resources/

- ClinGen system map



http://interactive.clinicalgenome.org/clingen-resource-system-map/

Clinical Actionability

https://www.clinicalgenome.org/knowledge-curation/actionability/

- Develop clear and robust criteria to guide decisions regarding actionable secondary findings
- Focus on findings associated with specific therapeutic or surveillance interventions in pre-symptomatic individuals
  1. Define elements of actionability
  2. Standardize evidence reviews
  3. Score gene-disease pairs with a semi-quantitative actionability metric

Katrina Goddard

Jim Evans

GenomeConnect

https://www.clinicalgenome.org/genomeconnect/

- **Collects** patient-entered phenotypic information and genetic testing reports through PatientCrossroads registry platform
- **Transfers** associated phenotypic and genotypic data into ClinGen-hosted database
- **Connects** participants with other families/individuals with same genetic variant(s) and researchers

GenomeConnect
The ClinGen Patient Portal

Andy Faucett

Brianne Kirkpatrick

Clinical Validity

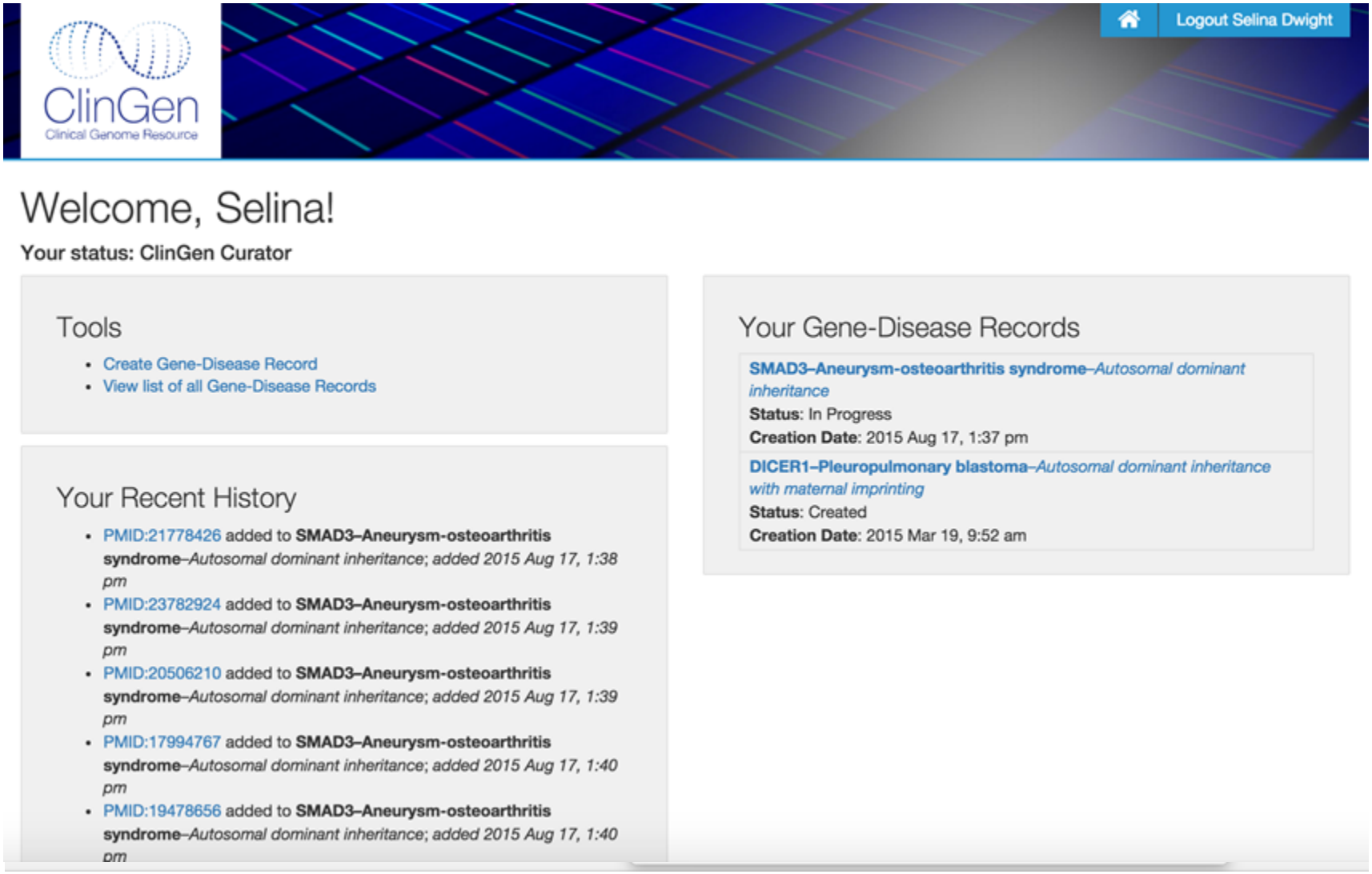
- ClinGen's Gene Curation WG has developed a **clinical validity classification** for assessing which genes play a role in disease diagnosis, prognosis, and drug response.
- Publication describing clinical validity framework expected late 2015
- **More details in presentation by Erin Riggs**

Clinical Validity Curation Interface

- Stanford is developing a curation interface to assist with the analysis of gene-disease pairs
  - Prototype of interface released this month
  - Beta version in October 2015

- Iterative improvements with feedback from ClinGen working groups
  - The Stanford development team expects this system to be accessible by community curators in 2017

Clinical Validity Curation Interface



SMAD3 – Aneurysm-osteoarthritis syndrome

Autosomal dominant inheritance

SMAD3
HGNC Symbol: SMAD3
NCBI Gene ID: 4088

Aneurysm-osteoarthritis syndrome
Orphanet ID: ORPHA284984
OMIM ID: 613795 [Edit]

Status: In Progress
Creator: Tam Sneddon – 2015 Sep 25, 12:14 pm
Participants: Selina Dwight
Last edited: Selina Dwight — 2015 Sep 29, 12:48 pm

Gene-Disease Record Variants

Click a variant to View, Curate, or Edit/Assess it. The icon indicates curation by one or more curators.

30306 | 30307

Add New PMID(s)

novel SMAD3 mutation: further delineation of the phenotype. **2013** May;161A(5):1028-35. doi: 10.1002/ajmg.a.35852. Epub 2013 Mar 29

PMID: 23554019

van de Laar IM et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. **2011** Feb;43(2):121-6. doi: 10.1038/ng.744. Epub 2011 Jan 9

PMID: 21217753

van de Laar IM et al. Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome **2012** Jan;49(1):47-57. doi: 10.1136/jmedgenet-2011-100382

PMID: 22167769

van de Laar IM et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. *Nat Genet.*. **2011** Feb;43(2):121-6. doi: 10.1038/ng.744. Epub 2011 Jan 9

PubMed

Abstract

Thoracic aortic aneurysms and dissections are a main feature of connective tissue disorders, such as Marfan syndrome and Loeys-Dietz syndrome. We delineated a new syndrome presenting with aneurysms, dissections and tortuosity throughout the arterial tree in association with mild craniofacial features and skeletal and cutaneous anomalies. In contrast with other aneurysm syndromes, most of these affected individuals presented with early-onset osteoarthritis. We mapped the genetic locus to chromosome 15q22.2-24.2 and show that the disease is caused by mutations in SMAD3. This gene encodes a member of the TGF-β pathway that is essential for TGF-β signal transmission. SMAD3 mutations lead to increased aortic expression of several key players in the TGF-β pathway, including SMAD3. Molecular diagnosis will allow early and reliable identification of cases and relatives at risk for major cardiovascular complications. Our findings endorse the TGF-β pathway as the primary pharmacological target for the development of new treatments for aortic aneurysms and osteoarthritis.

Evidence for PMID:21217753

Group

Family

Family 1
Selina Dwight
2015 Sep 29, 3:12 pm
No associations
Variants: 30306
View | Edit
Add new Individual to this Family

Family 2
Selina Dwight
2015 Sep 29, 3:18 pm
No associations
Variants: 30307
View | Edit
Add new Individual to this Family

Individual

prob-Family 1
Selina Dwight
2015 Sep 29, 3:12 pm
Associations: Family 1
Variants: 30306
View | Edit

prob-Family 2
Selina Dwight
2015 Sep 29, 3:18 pm
Associations: Family 2
Variants: 30307
View | Edit

Associated Variants

Curate Variants from the "Gene-Disease Record Variants" section above.

VariationId: 30306
Selina Dwight
2015 Sep 29, 2:58 pm
Associations: Family 1, prob-Family 1

VariationId: 30307
Selina Dwight
2015 Sep 29, 3:18 pm
Associations: Family 2, prob-Family 2

SMAD3 – Aneurysm - osteoarthritis syndrome

Autosomal dominant inheritance

Current Summary & Provisional Classification
Current Summary
Generated: 2015 Sep 29, 12:18 pm

Total Score: 15 (Strong)
Provisional Classification: Definitive [Edit Classification]

Generate New Summary

SMAD3
HGNC Symbol: SMAD3
NCBI Gene ID: 4088

Aneurysm - osteoarthritis syndrome
Orphanet ID: ORPHA284984
OMIM ID: 613795 [Edit]

Status: Summary/Provisional Classifications
Creator: Tam Sneddon – 2015 Aug 13, 10:23 am
Participants: Selina Dwight
Last edited: Selina Dwight — 2015 Aug 24, 5:56 am

Curation Summary and Provisional Classification

New calculation and Classification

The calculated values below are based on the set of saved evidence that exists when the "Generate New Summary" is clicked. To save these values as the "Current Summary & Provisional Classification" calculated values and make any changes to the Provisional Classification, you must click the Save button below.

Total Score: 18

Scoring Details:

Evidence	Count	Score
Final Experimental Score		**5**
Expression	1	0.5
Protein Interactions	1	0.5
Biochemical Function	0	0
Functional Alteration	0	0
Model Systems	2	4
Rescue	0	0
Proband Score		**6**
Number of probands with variants assessed as "supports" pathogenicity	16	
Publication Score		**5**
Clinical Publicatons	6	
Time Score (First Clinical Report)		**2**
Number of years since first report	4	

Calculated Clinical Validity Classification: Definitive

Selecte Provisional Clinical Validity Classification: Definitive

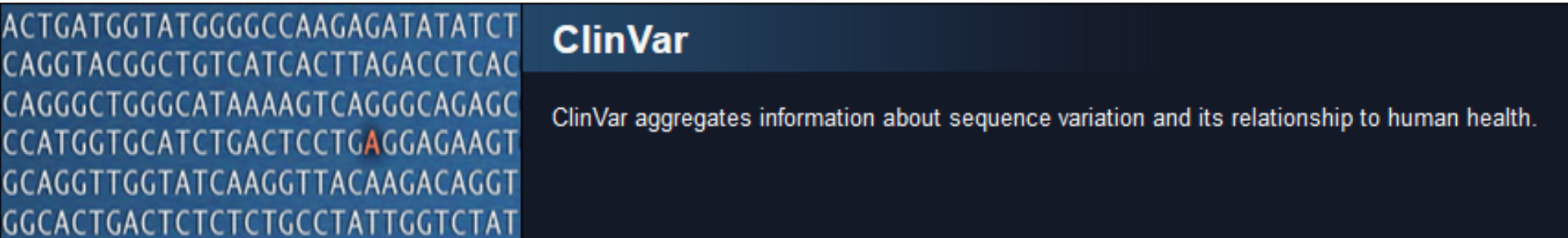
Explain Reason(s) for Change:

**Note: If your selected Clinical Validity Classification is different from the Calculated value, provide a reason to expain why you changed it.

Cancel Save

Variant Assessment

- ClinGen is utilizing the new ACMG sequence variant interpretation guidelines for assessment of variant pathogenicity
  - **More details in presentation by Heidi Rehm**
- ClinGen is also working with ClinVar to:
  - Encourage data submission
  - Resolve variant discrepancies
  - Define the review level of a submission
  - Review Expert Panel submissions



Pathogenicity Calculator

- Uses curated and derived evidence and then applies the ACMG rules to compute a preliminary conclusion

More details in presentation by Aleks Milosavljevic

ClinGen Pathogenicity Calculator

Logout

Allele Information

Property	Value
Allele ID	REG-CSER01-AL
Reference	NM_005228.3
HGVS	NM_005228.3(EGFR):c.2369C>T(p.Thr790Met)
Assembly	[No Data]

Guidelines - Conclusions

Apply Guidelines | View Evidence Doc

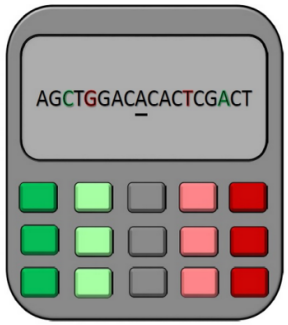
Conclusion	Unmet Condition	Rules
LikelyPathogenic	0	Pathogenic.Moderate ==2 & Pathogenic.Supporting >=2
Benign	1	Benign.Stand Alone ==1
Pathogenic	1	Pathogenic.Moderate ==2 & Pathogenic.Strong ==1 & Pathogenic.Supporting >=2 Pathogenic.Supporting >=2 & Pathogenic.Very Strong ==1 Pathogenic.Moderate >=2 & Pathogenic.Very Strong ==1
Uncertain Significance	1	Benign.Stand Alone >=1 & Pathogenic.Supporting >=1 Benign.Stand Alone >=1 & Pathogenic.Moderate >=1

Pathogenicity Evidence

Gene Name: EGFR Variant:	Benign			Pathogenic			
	Supporting	Strong	Stand Alone	Supporting	Moderate	Strong	Very Strong
POPULATION DATA					1		
COMPUTATIONAL AND PREDICTIVE DATA				1			
FUNCTIONAL DATA					1		
SEGREGATION DATA				1			
DE NOVO DATA							
ALLELIC DATA							
OTHER DATABASE				1			
OTHER DATA							

CLINGEN PATHOGENICITY CALCULATOR

ClinGen



WHAT IS THE CLINGEN PATHOGENICITY CALCULATOR?

The shift from genetic testing of individual genes to exome and genome sequencing has been accompanied by new challenges in genome interpretation. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology have published Standards and Guidelines for the Interpretation of Sequence Variants. To enable wide application of these and related standards and the development of collective knowledge by the community, ClinGen Resource project has developed ClinGenKB and associated resources. One ClinGenKB resource is the ClinGen Pathogenicity Calculator that enables evaluation of pathogenicity according to the ACMG/ACP Guidelines. The Calculator assists in making assertions based on evidence and rules such as those in ACMG guidelines for evaluating pathogenicity of genetic variants. It also provides visually appealing summary of evidence with drill-downs to supporting data. Evidence items can be added/edited/deleted interactively and conclusions re-calculated.

ClinGen Acknowledgements

ClinGen Steering Committee

Jonathan Berg, UNC
Lisa Brooks, NHGRI
Carlos Bustamante, Stanford
Mike Cherry, Stanford
James Evans, UNC
Andy Faucett, Geisinger
Andy Freedman, NCI

Katrina Goddard, Kaiser Permanente
Danuta Krotoski, NICHD
Melissa Landrum, NCBI
David Ledbetter, Geisinger
Christa Lese Martin, Geisinger
Aleks Milosavljevic, Baylor
Kelly Ormond, Stanford

Sharon Plon, Baylor
Erin Ramos, NHGRI
Heidi Rehm, Harvard
Steve Sherry, NCBI
Michael Watson, ACMG
Kirk Wilhelmsen, UNC
Marc Williams, Geisinger

Program Coordinators:
Danielle Azzariti, Miranda Hallquist, Brianne Kirkpatrick, Jules Koenig, Kristy Lee, Laura Milko, Annie Niehaus, Erin Riggs, Andy Rivera, Cody Sam, Meredith Weaver, Kira Wong

ClinGen Working Groups (WG)

Genomic Variant WG

Chairs: Christa Martin, Sharon Plon, Heidi Rehm

Informatics WG

Chair: Carlos Bustamante

Data Model WG

Chairs: Larry Babb

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

Phenotyping WG

Chair: David Miller

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

The ClinGen Gene Curation Process

Erin Rooney Riggs, MS, CGC
Geisinger Health System
ClinGen Gene Curation Working Group

Clinical validity

- A test's ability to "consistently and accurately detect or predict the outcome of interest"*
- Requires correctly identifying the causative variant within the *appropriate gene*
- How strong is the evidence that variation in that gene causes the disease in question?

*Haddow, J., Palomacki, G. ACCE: A Model Process for Evaluating Data on Emerging Genetic Tests. in *Human Genome Epidemiology: A Scientific Foundation for Using Genetic Information to Improve Health and Prevent Disease* (ed. Khoury, M., Little, J., Burke, W.) 217-233 (Oxford University Press, 2003).

ClinGen Clinical Validity Classifications

DEFINITIVE

STRONG

MODERATE

LIMITED

NO EVIDENCE REPORTED

CONFLICTING EVIDENCE REPORTED

See handout for detailed information. Explanatory video also available at:
http://calculator.clinicalgenome.org/ashg-2015

Assigning a Clinical Validity Classification

- Need to be able to summarize key data in a systematic and concise manner

- Key Data:
  - # Probands
  - # of Clinical Publications
  - Experimental Evidence
  - Time passed since initial gene-disease association
  - Presence or Absence of compelling contradictory evidence

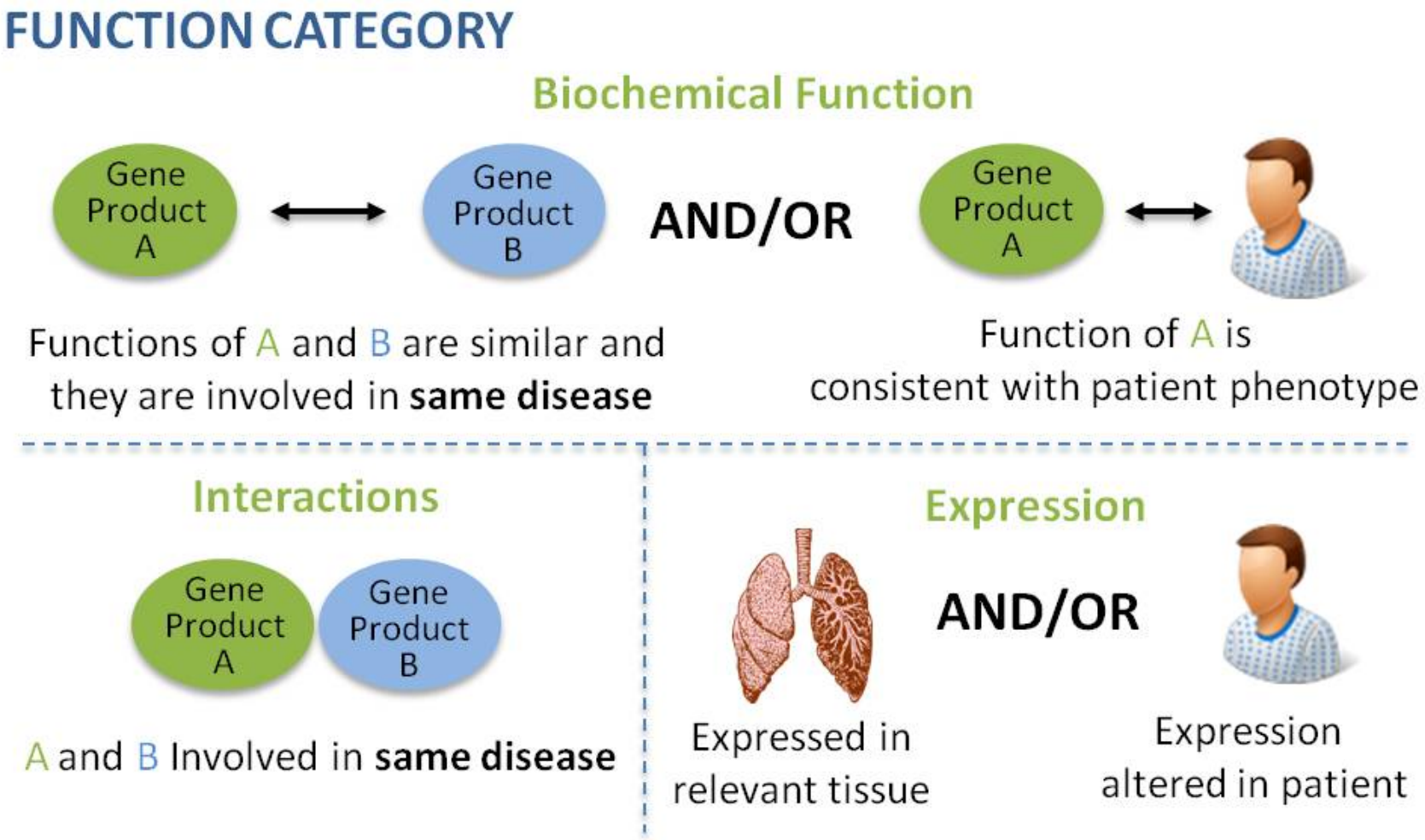
ClinGen Clinical Validity Summary Matrix

Assertion criteria	Description	Number of Points							
		0	1	2	3	4	5	6	7
# Probands	Total # of *unrelated* probands with variants that provide convincing evidence for disease causality across all curated literature	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	
Description of Contradictory Evidence:		**Limited:**	**0-8**	**Assertion:**
		Moderate:	**9-12**	
		Strong:	**13-16**	
		Definitive:	**17-20**	

See handout for detailed information. Explanatory video also available at: http://calculator.clinicalgenome.org/ashg-2015

Experimental Evidence



Consistent with MacArthur et al. Nature. 2014 Apr 24;508(7497):469-76

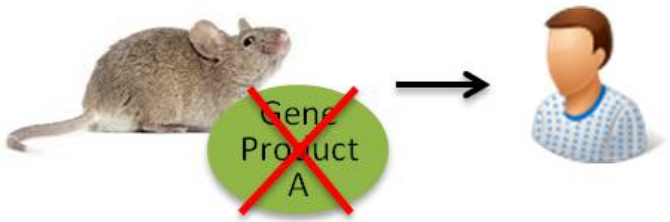
Experimental Evidence (cont'd)

FUNCTIONAL ALTERATION CATEGORY

Gene or gene product function is demonstrably altered in patients carrying candidate mutations

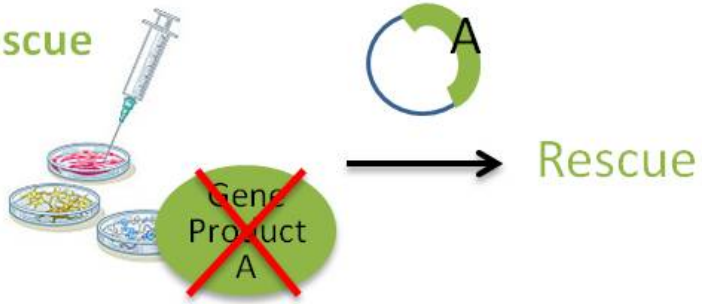
MODEL SYSTEMS & RESCUE CATEGORY

Model Systems



non-human animal or cell-culture models with a similarly disrupted copy of the affected gene show a phenotype consistent with human disease state.

Rescue



the cellular phenotype in patient-derived cells or engineered equivalents can be rescued by addition of the wild-type gene product

Consistent with MacArthur et al. Nature. 2014 Apr 24;508(7497):469-76

Experimental Evidence Scoring -> Max Score 6*

Evidence Category	Evidence Type	Score Range	Suggested points/ evidence	Max Score
Function	Biochemical Function	0-2	½ for each piece of evidence in any category	2
	Protein Interaction	0-2		
	Expression	0-2		
Functional Alteration	Patient cells	1-2	1	2
	Non-patient cells	0-1	½	
Models and Rescue	Animal model	2-4	2	4
	Cell culture model	0-2	1	
	Rescue	2-4	2	
			Total Final Score	**0-8**

***The total number of available experimental "points" is 8 to allow for flexibility in the types of evidence that are combined to achieve a maximum score of 6 in the matrix.**

Examples

NHP2 and Dyskeratosis Congenita

Dyskeratosis Congenita (DC)	
Characteristics	Defective tissue maintenance, impaired stem cell function, cancer predisposition
Cause	Shortened telomeres resulting from a defect in telomerase
Clinical Features (includes)	• Leukoplakia • Nail dystrophy • Reticular pigmentation • Pancytopenia • Lung fibrosis • etc.

NHP2 is part of the telomerase enzyme complex

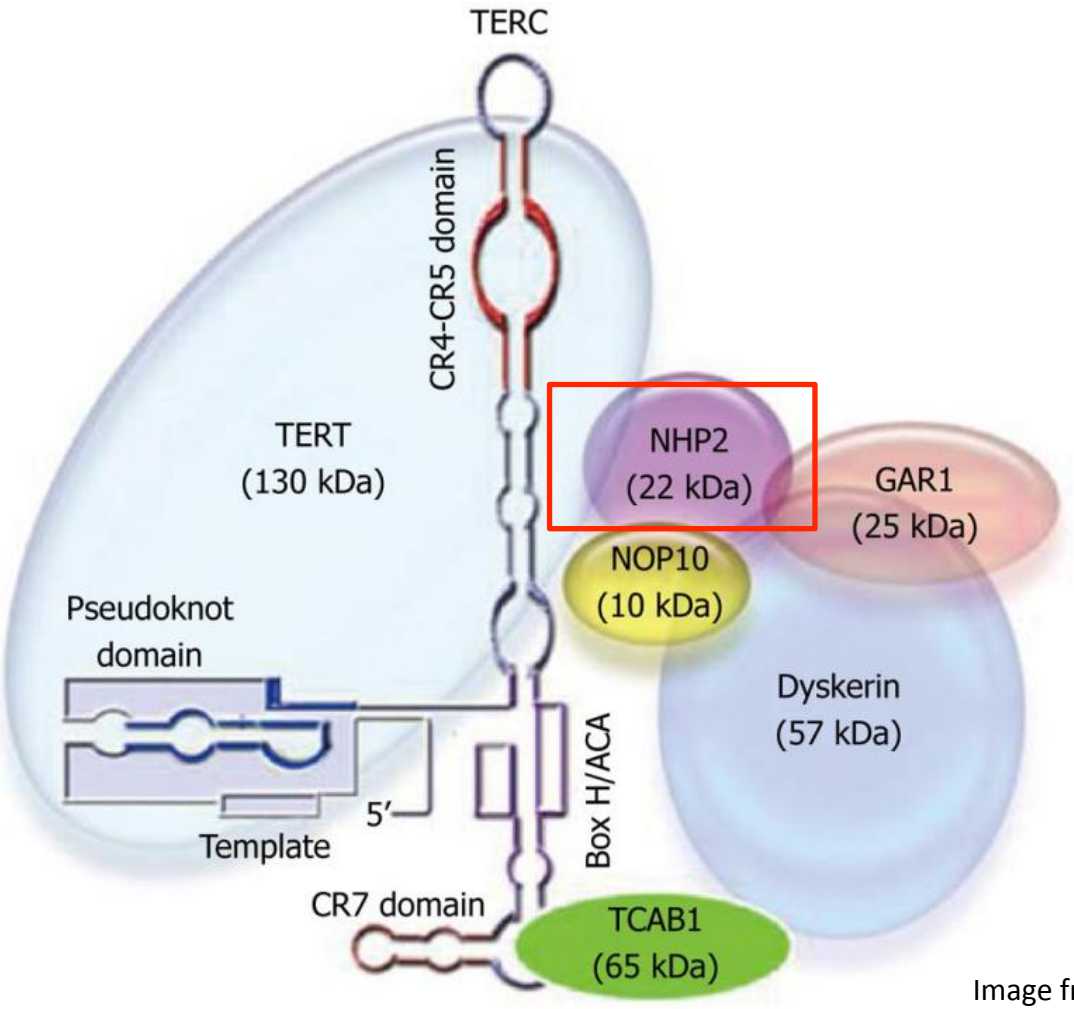


Image from: World J Stem Cells. 2011 October 26; 3(10): 89-95.

Probands: Single Clinical Report (Vulliamy et al., 2008); Two Unrelated Turkish Probands

	Variants	Findings	Inheritance	Control Information
Patient 1	Homozygous c.415T>C (p.Tyr139His)	nail dystrophy, thrombocytopenia, testicular atrophy, opportunistic infections, growth and mental retardation, liver cirrhosis, and intracranial calcification; shortened telomeres	Heterozygous unaffected parents; reportedly unrelated	Not detected in 282 controls of mixed ethnic origin or in 98 Turkish individuals
Patient 2	Compound Heterozygous c.376 G>A (p.Val126Met)/ c. 460T>A (p.X154ArgextX*52)	nail dystrophy , leucoplakia, reticulate skin pigmentation, peripheral pancytopenia, progressive bone marrow failure; shortened telomeres	same	same

Experimental Evidence

Category	Evidence_Type	Description	Given	Max
Function	Biochemical Function	*NHP2*: part of the telomerase RNP complex (PMID: 11074001)	**0.5**	2
	Protein Interaction	None curated.	**-**	
	Expression	None curated.	**-**	
Gene Disruption	Gene Disruption	*NHP2* knockdown resulted in reduction in TERC levels observed in patient material (PMID:18523010)	**1**	2
Models & Rescue	Model Systems	None curated.	**-**	4
	Rescue	Expression of wild type *NHP2* increases TERC accumulation compared with cells with exogenous mutant *NHP2* (PMID: 18523010)	**1**	
Total			**2.5 → 3**	

NHP2-Dyskeratosis Congenita Summary

		Number of Points							
		0	1	2	3	4	5	6	7
Assertion Criteria	# of probands with with variants that provide a compelling etiology for their phenotype	0	2	4-6	7-9	10-12	13-15	16-18	19+
	# of Points for Experimental data	0	1	2	3	4	5	6+	
	# of independent publications with cases supporting the association	0	1	2	3	4	5+		
	Time since first publication (if 2 or fewer publications linking the gene to disease exist, then 1 is the highest score that can be assigned to time)	this yr	1-3 yr	≥3 yr					
Contradictory Evidence		NO							
Description		N/A							

Assertion	Total Score
Limited:	0-8
Moderate:	9-12
Strong:	13-16
Definitive:	17-20

Total Score: 6

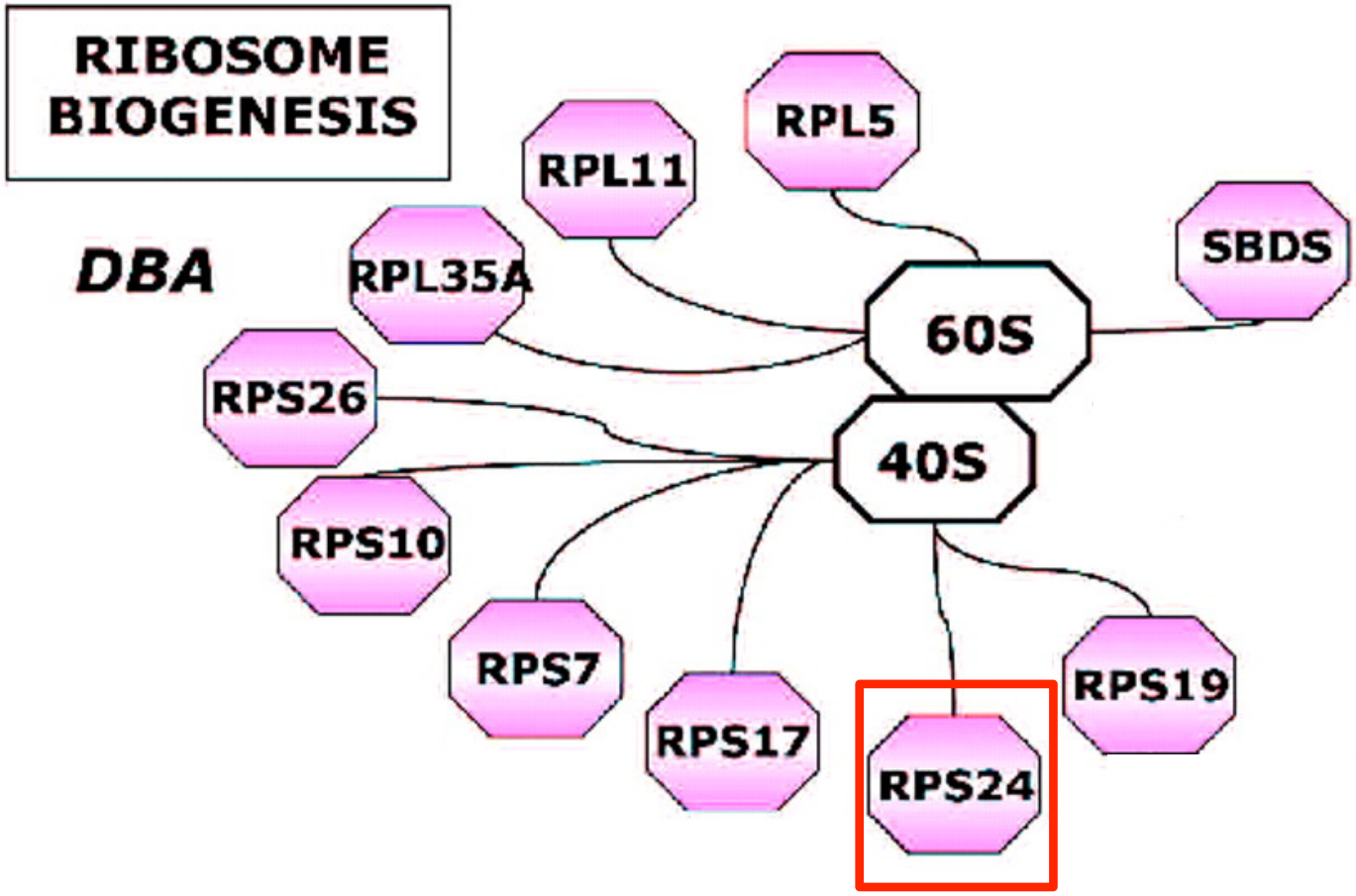
Preliminary Classification: Limited

RPS24 and Diamond-Blackfan Anemia (DBA)

Diamond Blackfan Anemia (DBA)

From OMIM:	
General	Inherited red blood cell aplasia that usually presents in the first year of life
Cause	Disorder of ribosome biogenesis
Clinical Features (includes)	• Normochromic macrocytic anemia • Reticulocytopenia • Nearly absent erythroid progenitors in bone marrow • Some pts with: growth retardation, congenital anomalies of the heart, upper limb, urinary system, etc. • Increased MCV; elevated eADA, HbF

RPS24



Adapted from: Inderjeet Dokal and Tom Vulliamy, Haematologica 2010;95:1236-1240

haematologica
the hematology journal

5 Unrelated Probands; 3 Clinical Publications

PMID	Author, Year	Variant	Age Diagnosed	Family Testing	Control Information
17186470	Gazda, 2006	Heterozygous Gln106Ter	N/A	5 family members with mutation, but only 3 have clinical features	Not found in 220 control individuals
17186470	Gazda, 2006	Heterozygous Arg162Ter	N/A	sporadic	Not found in 220 control individuals
17186470	Gazda, 2006	Heterozygous Del 22aa	N/A	Found in proband and father (who was affected in childhood)	Not found in 220 control individuals
19773262	Quarello, 2010	c.64_66delCAA → del Gln22 was identified in a patient without somatic malformations and in clinical remission at last follow-up.	N/A	*de novo*	Not found in 100 controls
23812780	Landowski, 2013	Heterozygous Deletion of exons 1-3	N/A	*de novo*	No CNV in 3 controls

Experimental Evidence

Category	Evidence_Type	Description	Given	Max
Function	Biochemical Function	*RPS24* functions in the maturation of the 5'-ETS (PMID: 18230666)	**0.5**	2
	Protein Interaction	*RPS24* interacts with other ribosomal proteins, which are associated with DBA (PMID: 22939629)	**0.5**	
	Expression	Reduced mRNA expression and protein expression of *RPS24* in patient cell lines (PMID: 17186470)	**0.5**	
Gene Disruption	Gene Disruption	•Patient cell lines show a clear alteration of pre-rRNA processing by Northern blot •KD of *RPS24* in HeLa cells shows that *RPS24* is essential in forming the small ribosomal subunit	**2**	2
Models & Rescue	Model Systems	None curated.	**-**	4
	Rescue	None curated.	**-**	
Total			**3.5 → 4**	

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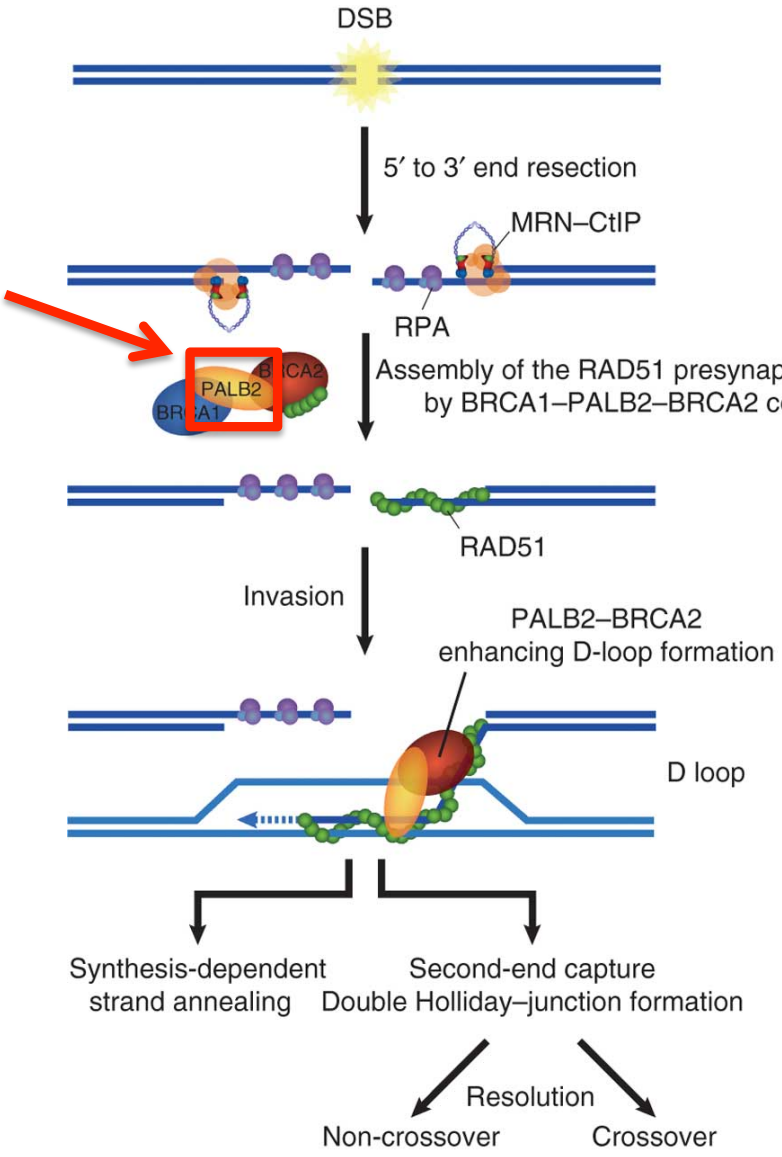
RPS24: DBA Summary Matrix

Assertion criteria	Description	Number of Points							
		0	1	2	3	4	5	6	7
# Probands	Total # of unrelated probands with variants that provide a compelling etiology for their phenotype across all curated literature	N/A	1-3	**5**	7-9	10-12	13-15	16-18	19+
Functional evidence points	Points given based on the **gene-level** functional evidence supporting a role for this gene in disease	0	1	2	3	**4**	5	6+	
# Independent Publications	# of Independent publications identifying **human** variants in the gene in association with disease	N/A	1	2	**3**	4	5+		
Time	# of years since first publication reporting a disease association **(if ≤2 publications --> then 1 is max score for time)**	this yr	1-3 yr	**9 yrs**					
Is there valid contradictory evidence?		No	**Classification**		**Total Score**	**Total Score: Assertion:**			
Description:	**N/A**		**Limited:**		**0-8**				
			Moderate:		**9-12**				
			Strong:		**13-16**				
			Definitive:		**17-20**				

Total Score: 10 Assertion: Moderate

PALB2 and Hereditary Breast Cancer

PALB2



Buisson et al. 2010, Nat Struc & Molec Biol 17:1247-1254

Relevant Clinical Evidence

Reference	Mutation(s)	OR for HBC	# BC probands tested	# Controls
Rahman et al. 2007	Mult. Truncating variants	2.3 (p = 0.0025)	923 (10 w/ mut)	1084
Erkko et al. 2007	(c.1592delT, p.Leu531Fs)	11.3 (p = 0.005) 3.94 (p = 0.003)	113 (3 with mut) 1,918 (18 w/ mut)	2401 (6 with mut)
Tischkowitz et al. 2007	c.229delT, p.C77fs	N/A –segregation and other functional evidence support variant	119 (1 w/ mut)	N/A

and more…

Experimental Evidence

Category	Evidence_Type	Description	Given	Max
Function	Biochemical Function	• *PALB2* enables nuclear functions of *BRCA2* (16793542) • *BRCA2* mutations that disrupt the interaction with *PALB2* cause breast cancer (16793542)	**0.5**	2
	Protein Interaction	*PALB2* interacts with *BRCA2* (16793542)	**0.5**	
	Expression	None curated.	**-**	
Gene Disruption	Gene Disruption	*PALB2* frameshift results in loss of function (no longer binds *BRCA2* or properly undergoes HR) (17287723)	**2**	2
Models & Rescue	Model Systems	*Palb2*-deficient murine ES cells recapitulate DNA damage caused by *PALB2* depletion in human cells (23657012)	**2**	4
	Rescue	None curated.	**-**	
Total				**5**

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Summary and Assertion

Assertion criteria	Description	Number of Points							
		0	1	2	3	4	5	6	7
# Probands	Total # of unrelated probands with variants that provide a compelling etiology for their phenotype across all curated literature	N/A	3	4-6	7-9	10-12	13-15	16-18	**32+**
Functional evidence points	Points given based on the **gene-level** functional evidence supporting a role for this gene in disease	0	1	2	3	4	**5**	6+	
# Independent Publications	# of Independent publications identifying **human** variants in the gene in association with disease	N/A	1	2	**3+**	4	5+		
Time	# of years since first publication reporting a disease association **(if ≤2 publications --> then 1 is max score for time)**	this yr	1-3 yr	**8 yrs**					
Is there valid contradictory evidence?		N	**Classification**		**Total Score**	**Score:**			
Description:	**N/A**		**Limited:**		**0-8**	**Assertion:**			
			Moderate:		**9-12**				
			Strong:		**13-16**				
			Definitive:		**17-20**				

Total Score: 17 Assertion: Definitive

ClinGen Gene Curation Working Group

WG Chairs:

- Jonathan Berg
- Christa Lese Martin

Gene Curation Small Group:

- Ozge Birsoy
- Adam Buchanan
- Selina Dwight
- Raj Ghosh
- Erin Rooney Riggs
- Tasha Strande
- Tam Sneddon

Other WG Members:

- Danielle Azzariti
- Matt Ferber
- Birgit Funke
- Monica Giovanni
- Katrina Goddard
- Steven Harrison
- Laura Milko
- Mike Murray
- Annie Niehaus
- Julianne O'Daniel
- Sharon Plon
- Erin Ramos
- Andy Rivera
- Heidi Rehm
- Avni Santani
- Alan Scott
- Bryce Seiffert
- Mike Watson
- Meredith Weaver
- Bob Wildin
- Dane Witmer
- Kira Wong

Questions?

Email eriggs@geisinger.edu or clingen@clinicalgenome.org

Standardizing Variant Classification and Resolving Differences

Heidi L. Rehm, PhD, FACMG

Director, Partners Healthcare Laboratory for Molecular Medicine
Clinical Director, Broad Institute Clinical Research Sequencing Platform
Associate Professor of Pathology, Brigham and Women's Hospital and Harvard Medical School

PARTNERS HEALTHCARE | PERSONALIZED MEDICINE

BWH

HARVARD MEDICAL SCHOOL

BROAD INSTITUTE

Data Submission to ClinVar

Sharing Clinical Reports Project

Genome Connect and Free-the-Data

Researchers

Clinical Labs

Expert Groups

Clinics

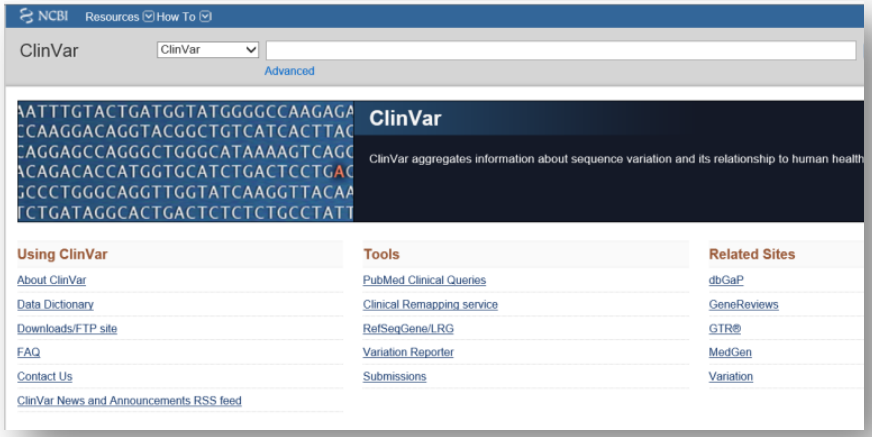
Patients

Patient Registries

Unpublished or Literature Citations

ClinVar
Variant-level Data

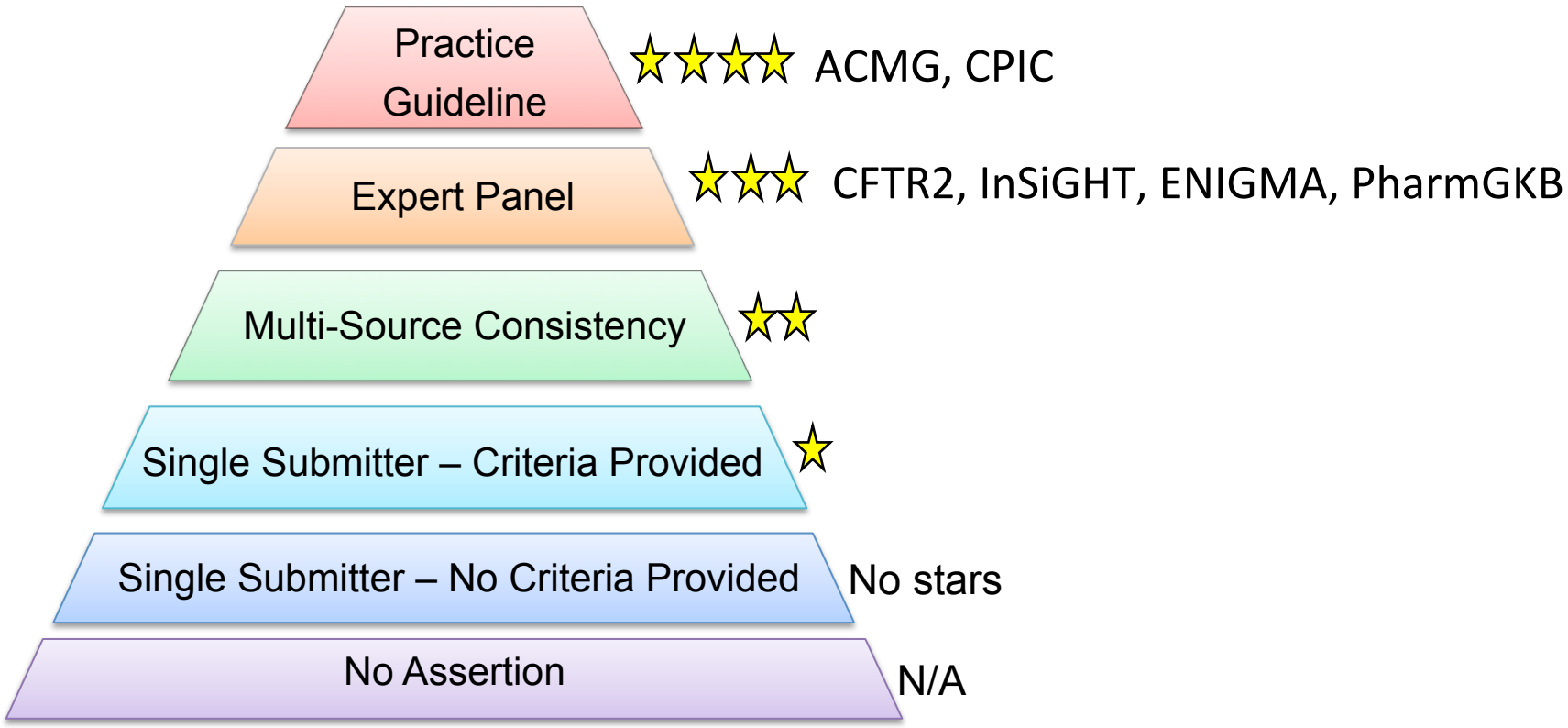
Linked Databases
- OMIM
- CFTR2
- BIC
- InSiGHT
- PharmGKB

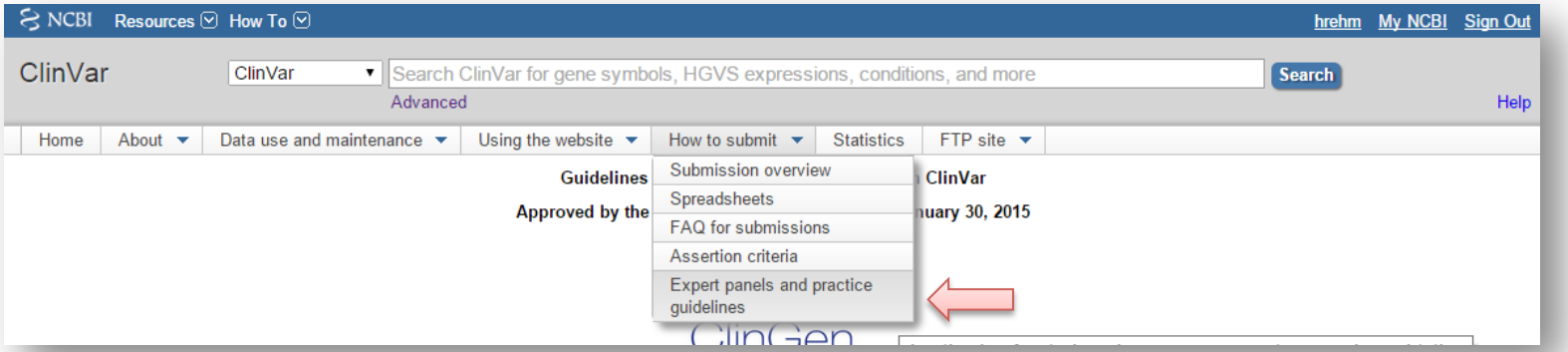


>382 ClinVar submitters
>160,470 submissions
>112,496 unique interpreted variants

ClinVar as of Oct 2nd, 2015

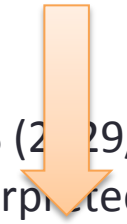
Assertion Levels in ClinVar





ClinVar Variant Interpretation Comparisons

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



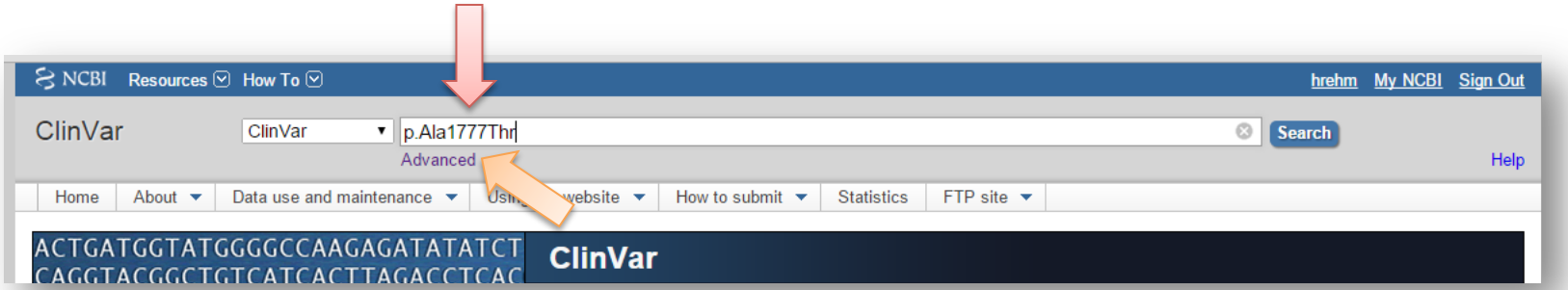
17% (2 29/12,895)
are interpr ted differently

ClinVar Data from May 4th, 2015

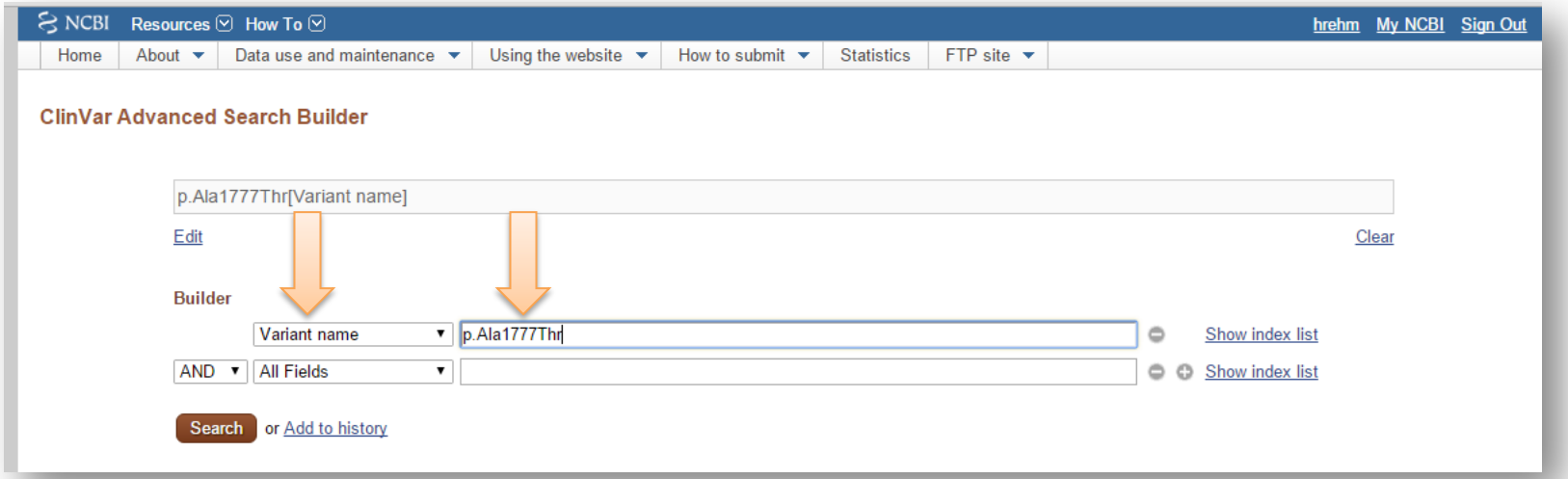
Rehm et al. ClinGen – The Clinical Genome Resource. NEJM. May 27th, 2015

Searching ClinVar

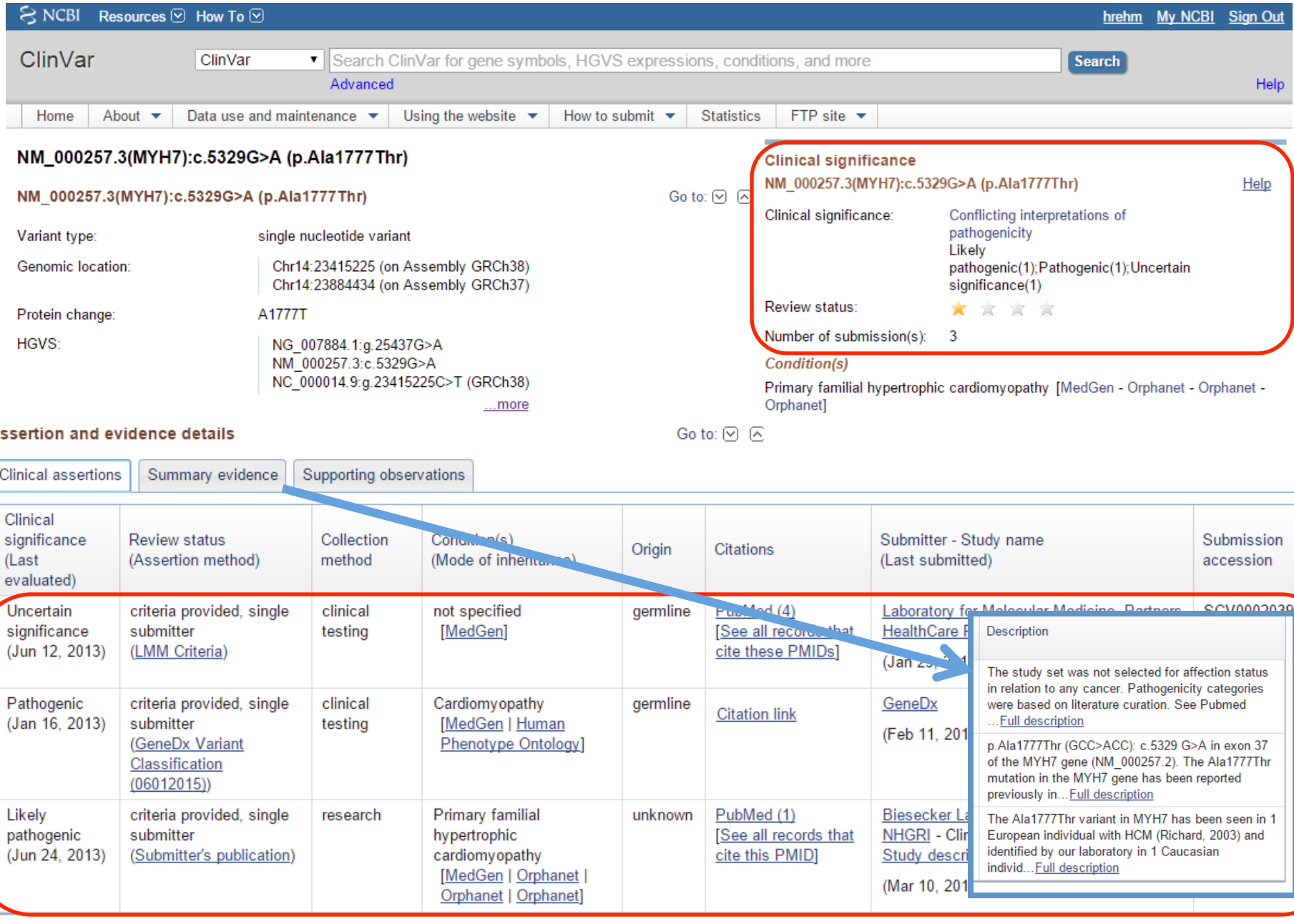
- Enter “p.Ala1777Thr” into Search field (or c. 5329G>A)



OR



ClinVar Variant View



ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

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, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; 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.[1] 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

	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 *BP4* Missense when 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	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 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*			

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
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

Monogenic disease terms

Pathogenic

Likely pathogenic

Uncertain significance (VUS)

Likely benign

Benign

Resolving differences in interpretation and applying the ACMG rules

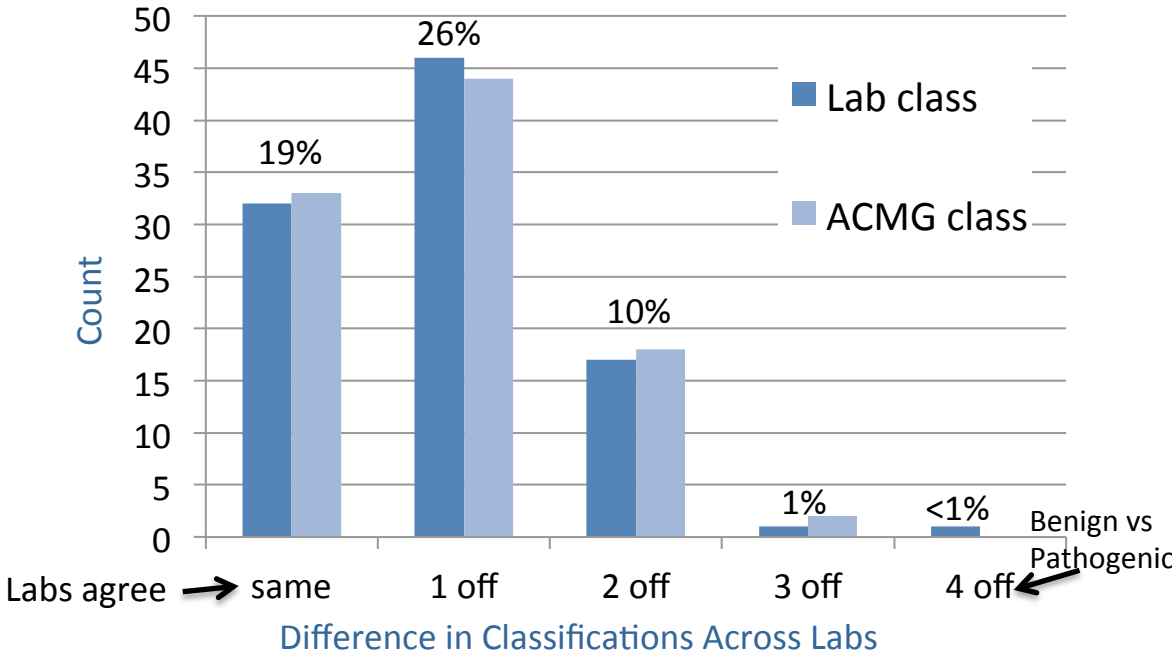
CSER Variant Bakeoff

9 sites, 11 variants submitted by each lab = 99 variants total

9 variants evaluated by all 9 sites and 90 variants by 3 sites – used both lab rules and ACMG rules

No statistically significant difference comparing rule sets

52% of differences resolved



Acknowledgements:
Laura Amendola, Heather McLaughlin, Gail Jarvik, Heidi Rehm
BASIC^3/Baylor - Yang Y, Ghosh R, Milosavljevic M, Plon SE
CanSeq/DFCI - Ghazani A, Van Allen E, Wagle N, Garraway L
ClinSeq/NIH - Biesecker L
Hudson-Alpha - Cooper G
MedSeq/BWH – McLaughlin H, Rehm H, Lebo M, Green RC
NCGenes/UNC - Strande NT, Berg JS, Evans JP
NextGen/Kaiser –Richards S, Punj S, Pak C, Akkari Y, Leo M, Goddard KAB
NextMed/UW – Amendola L, Hart R, Salama J, Horton C, Dorschner M, Jarvik G
PediSeq/CHOP - Conlin LK, Biswas S, Dulik M, Spinner N, Krantz I

ASHG 2015 Poster
1986-F

GLA c.639+919G>A; Fabry disease

- Reported in 6 individuals with a later-onset, cardiac variant of Fabry disease - all individuals had reduced GLA enzyme activity (Ishii 2002).
- Variant causes abnormal splicing with 57 bases added causing a truncation (Ishi 2002).
- In 94 adults (22 men + 72 women) found with variant, GLA activity was 10% of normal in the men and 50% of normal in the women. LVH was detected in 21% overall and 67% in the men (Lin 2010).
- Newborn screen of 110,027 newborns detected reduced GLA activity in 37 infants with the 639+919G>A variant. This study also evaluated 20 maternal grandparents of these infants and found that 3/9 grandfathers with this variant had HCM. Finally, 4/16 males who had been diagnosed with idiopathic HCM had reduced GLA activity in combination with the 639+919G>A variant.
- Variant absent from 528 race-matched controls (case-control statistical difference calculated in Lin 2010).

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GLA (NM_000169.2):c.639+919G>A; Fabry disease

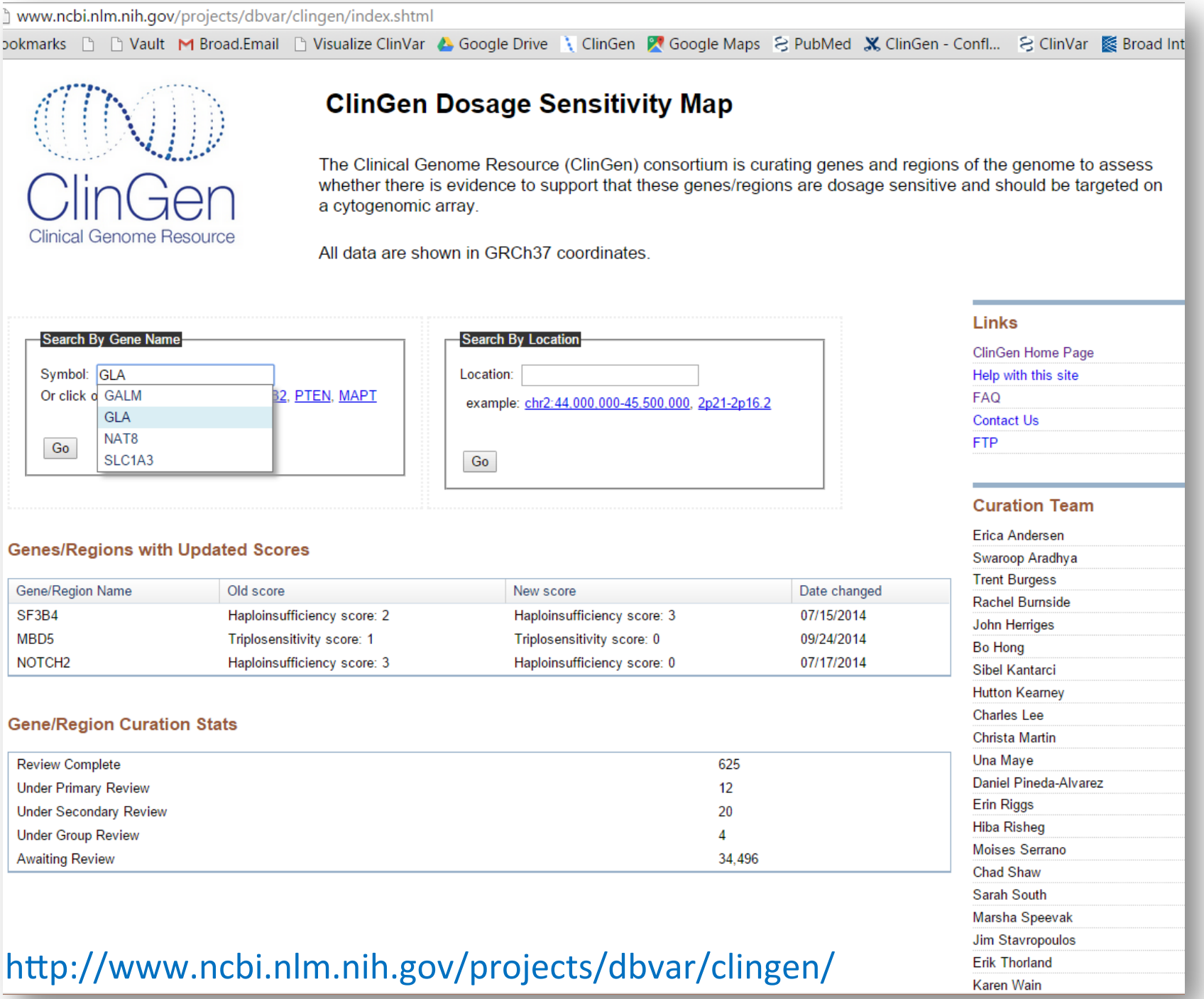
Site	Lab Rules	ACMG Rules	PVS1	PS3	PS4	PM4	PP1	PP5	PP3	BP4
Site 1	Pathogenic	Pathogenic	?	X	X		M			
Site 2	Pathogenic	Uncertain Significance		X		X	X	X		X
Site 3	Pathogenic	Likely Pathogenic		X			X		X	

PVS1 – Null variant

Yes, but reduce rule strength to "strong" due to reliance on functional assay to prove LOF

ACMG Rule:

PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease



http://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/

www.ncbi.nlm.nih.gov/projects/dbvar/clingen/clingen_gene.cgi?sym=GLA&subject=

ClinGen
Clinical Genome Resource

ClinGen Genome Curation Page

Links

ClinGen Curation Home Page
ClinGen Home Page
Help with this site
FAQ
Contact Us
Report information on a gene

GLA

Curation Status: Complete

id: ISCA-20553

Date last evaluated: 2012-05-17

Issue Type: ClinGen Gene Curation

Gene type: protein-coding

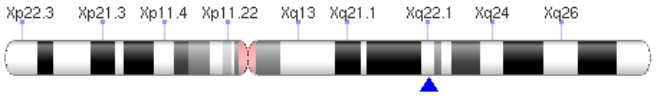
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene/2717

OMIM: http://omim.org/entry/300644

Gene Reviews: http://www.ncbi.nlm.nih.gov/books/NBK1292/?term=GLA

ClinGen Haploinsufficiency Score: 3

ClinGen Triplosensitivity Score: 0



Location Information

Xq22.1

GRCh37/hg19 chrX: 100,652,779-100,663,001

View: NCBI | Ensembl | UCSC

Print Full Report

Genome View | Evidence for Haploinsufficiency Phenotypes | Evidence for Triplosensitive Phenotypes

Haploinsufficiency score: 3
Strength of Evidence (disclaimer): Sufficient evidence for dosage pathogenicity
Haploinsufficiency phenotype: FABRY DISEASE
Haploinsufficiency phenotype comments: Loss of function mutations in GLA cause Fabry disease in males. Female carriers frequently manifest clinical features, usually with later onset. See GeneReviews.

The loss-of-function and triplosensitivity ratings for genes on the X chromosome are made in the context of a male genome to account for the effects of hemizygous duplications or nullizygous deletions. In contrast, disruption of some genes on the X chromosome causes male lethality and the ratings of dosage sensitivity instead take into account the phenotype in female individuals. Factors that may affect the severity of phenotypes associated with X-linked disorders include the presence of variable copies of the X chromosome (i.e. 47,XXY or 45,X) and skewed X-inactivation in females.

GLA (NM_000169.2):c.639+919G>A; Fabry disease

Site	Lab Rules	ACMG Rules	PVS1	PS3	PS4	PM4	PP1	PP5	PP3	BP4
Site 1	Pathogenic	Pathogenic	?	X	X		M			
Site 2	Pathogenic	Uncertain Significance		X		X	X	X		X
Site 3	Pathogenic	Likely Pathogenic		X			X		X	

PM4 - Protein length changing variant

No, only applicable for in-frame deletions, not a splice variant that leads to a frameshift

PP5 – Reputable source = pathogenic

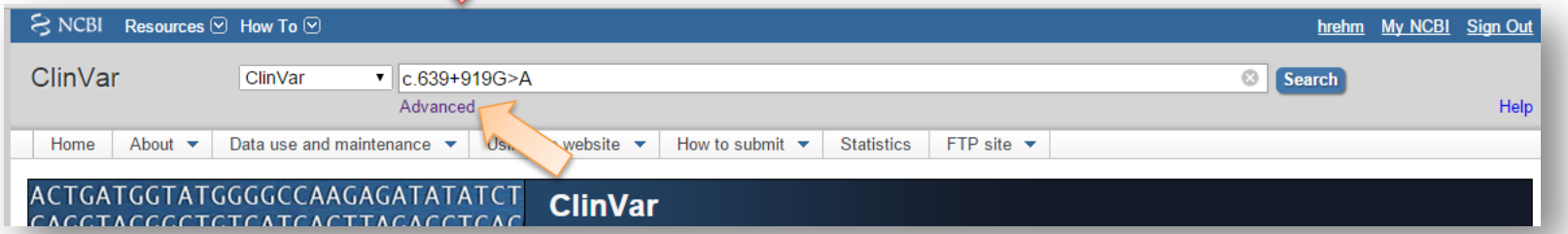
?

PP3, BP4 – Multiple lines of computational evidence

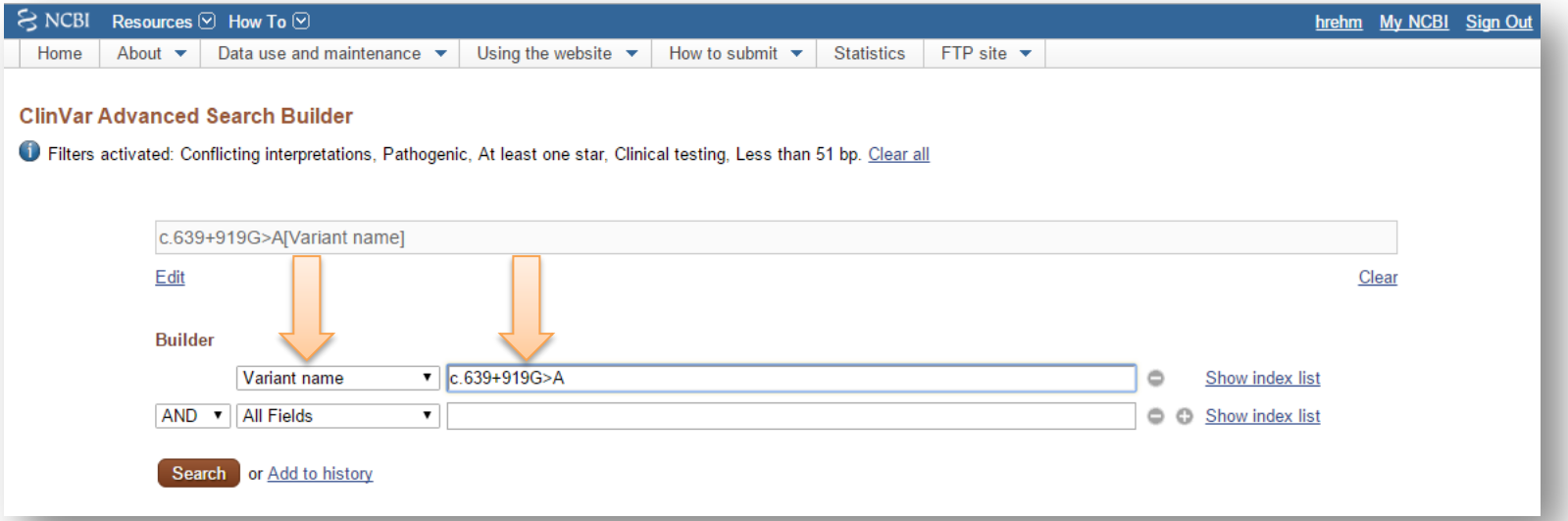
No (All programs must be consistent)

Search ClinVar

- Enter “c.639+919G>A” into Search field



OR



NM_000169.2(GLA):c.639+919G>A

NM_000169.2(GLA):c.639+919G>A Go to:

Variant type:	single nucleotide variant
Cytogenetic location:	Xq22.1
Genomic location:	ChrX:101399747 (on Assembly GRCh38) ChrX:100654735 (on Assembly GRCh37)
Other names:	IVS4, G-A, -4
HGVS:	NG_007119.1:g.13217G>A NM_000169.2:c.639+919G>A NC_000023.11:g.101399747C>T (GRCh38) NC_000023.10:g.100654735C>T (GRCh37) NM_000169.2:c.640-801G>A

Clinical significance

NM_000169.2(GLA):c.639+919G>A Help

Clinical significance: Pathogenic

Review status: ★ ☆ ☆ ☆

Number of submission(s): 2

Condition(s)

Fabry's disease [MedGen - Orphanet - OMIM]

Fabry disease, cardiac variant [MedGen]

See supporting ClinVar records

Full description for Laboratory for Molecular Medicine, Partners HealthCare Pers...

The c.639+919G>A variant in GLA (also referred to as c.640-801G>A) has been reported in 6 individuals with a later-onset, cardiac variant Fabry disease and in multiple individuals with HCM or LVH, all of which exhibited reduced GLA enzyme activity levels (Ishii 2002, Lin 2009, Lin 2010). Additionally, this variant was absent from 528 control chromosomes tested in two studies (Ishii 2002, Hwu 2009). This variant has been previously identified by our laboratory in 2 Asian individuals with HCM, and segregated in 3 affected relatives (2 with Fabry disease and 1 with reduced GLA activity). Finally, molecular studies demonstrated that this variant leads to abnormal splicing resulting in the introduction of an additional 57 nucleotides into the GLA transcript, ultimately leading to a truncated protein (Ischii 2002). In summary, the c.639+919G>A variant meets our criteria to be classified as pathogenic based on its presence in clinically affected individuals, absence from controls, segregation studies and functional evidence.

Assertion and evidence details

Go to:

Clinical assertions | Summary evidence | Supporting observations

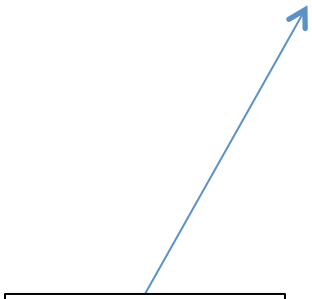
Germline

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 (Oct 7, 2014)	criteria provided, single submitter (LMM Criteria)	clinical testing	Fabry's disease (X-linked inheritance) [MedGen | Orphanet | OMIM]	germline	PubMed (8) [See all records that cite these PMIDs]	Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine (Jan 29, 2015)	SCV000203980
Pathogenic (Dec 8, 2010)	no assertion criteria provided	literature only	Fabry disease, cardiac variant [MedGen]	germline	PubMed (1) [See all records that cite this PMID]	OMIM (Dec 30, 2010)	SCV000031747

GLA (NM_000169.2):c.639+919G>A; Fabry disease

Site	Lab Rules	ACMG Rules	PVS1	PS3	PS4	PM4	PP1	PP5	PP3	BP4
Site 1	Pathogenic	Pathogenic	?	X	X		M			
Site 2	Pathogenic	Uncertain Significance		X		X	X	X		X
Site 3	Pathogenic	Likely Pathogenic		X			X		X	



PP5 – Reputable source = pathogenic

No – only use if evidence not available

GLA (NM_000169.2):c.639+919G>A; Fabry disease

Site	Lab Rules	Lab Rules	PVS1	PS3	PS4	PM4	PP1	PP3	PP5	BP4
Site 1	Pathogenic	Pathogenic	?	X	X		M			
Site 2	Pathogenic	Uncertain Significance		X		X	X		X	X
Site 3	Pathogenic	Likely Pathogenic		X			X	X		

PS3 – Fx studies

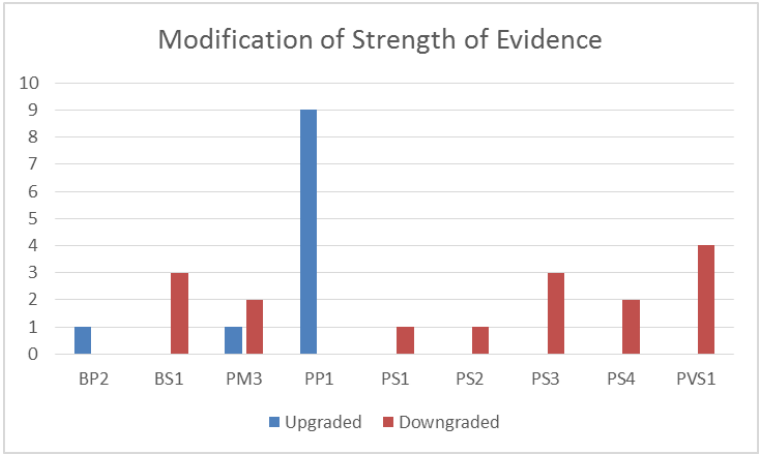
Yes, α-gal testing well-established

PS4 – Case>controls

Yes, at least one publication with a statistically sig p value. Other papers show statistical increase though one must calculate manually

PP1 – Segregation

Yes, one site increased to "moderate" due to 3 segregations



GLA (NM_000169.2):c.639+919G>A; Fabry disease

Site	Lab Rules	ACMG Rules	Post Discussion		PVS1	PS3	PS4	PM4	PP1	PP3	PP5	BP4
Site 1	Pathogenic	Pathogenic	Pathogenic		?	X	X		M			
Site 2	Pathogenic	Uncertain Significance	Pathogenic			X		X	X		X	X
Site 3	Pathogenic	Likely Pathogenic	Pathogenic			X			X	X		
Consensus	**Pathogenic**	**Pathogenic**	**Pathogenic**		**S**	**X**	**X**		X			
			Final		*	*	*		*			

PVS1 (Strong) – Null variant

PS3 – Fx studies

PS4 – Case>controls

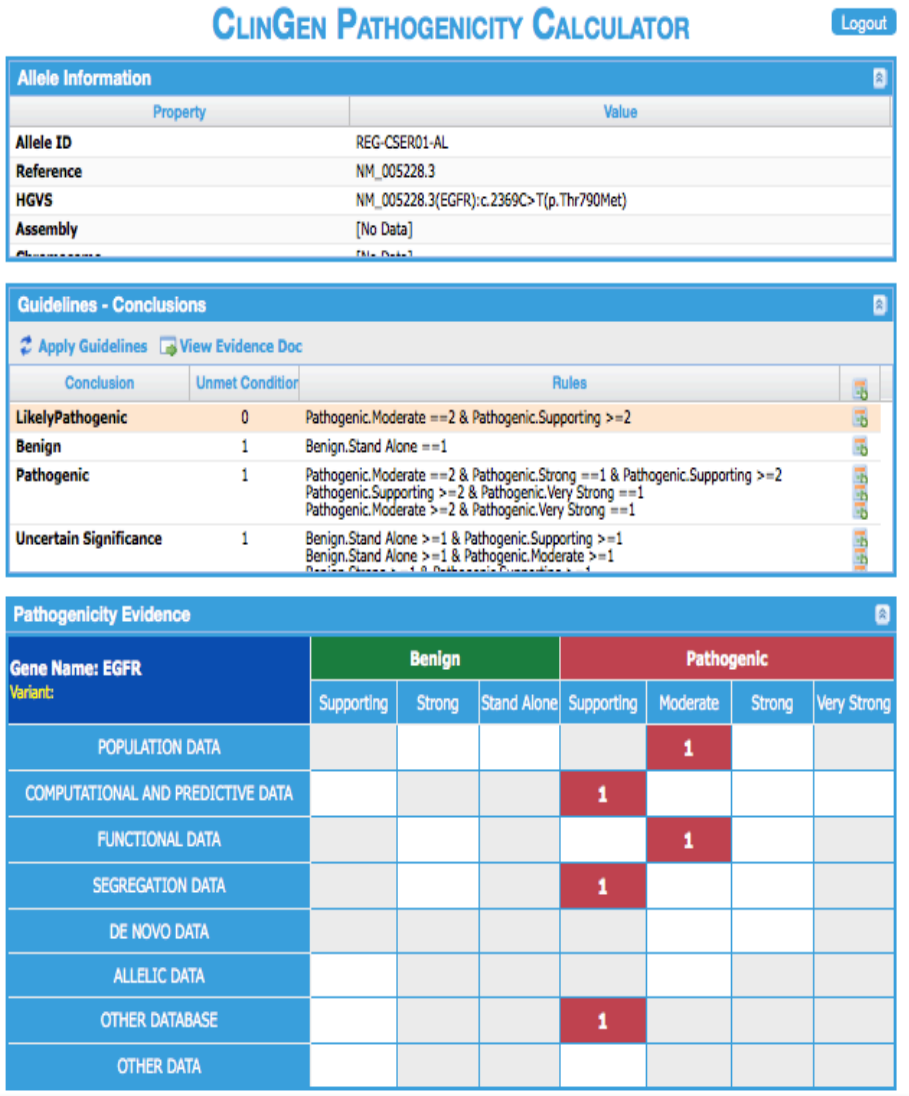
PP1 – Segregation

ACMG rules:
≥2 strong = Pathogenic

Consensus interpretation of all 3 sites: Pathogenic

For the CSER bakeoff, we observed several examples where the rules were added up incorrectly

A calculator is needed!



ClinGen Pathogenicity Calculator

Logout

Allele Information

Property	Value
Allele ID	REG-CSER01-AL
Reference	NM_005228.3
HGVS	NM_005228.3(EGFR):c.2369C>T(p.Thr790Met)
Assembly	[No Data]

Guidelines - Conclusions

Apply Guidelines | View Evidence Doc

Conclusion	Unmet Condition	Rules
LikelyPathogenic	0	Pathogenic.Moderate ==2 & Pathogenic.Supporting >=2
Benign	1	Benign.Stand Alone ==1
Pathogenic	1	Pathogenic.Moderate ==2 & Pathogenic.Strong ==1 & Pathogenic.Supporting >=2 Pathogenic.Supporting >=2 & Pathogenic.Very Strong ==1 Pathogenic.Moderate >=2 & Pathogenic.Very Strong ==1
Uncertain Significance	1	Benign.Stand Alone >=1 & Pathogenic.Supporting >=1 Benign.Stand Alone >=1 & Pathogenic.Moderate >=1

Pathogenicity Evidence

Gene Name: EGFR Variant:	Benign			Pathogenic			
	Supporting	Strong	Stand Alone	Supporting	Moderate	Strong	Very Strong
POPULATION DATA					1		
COMPUTATIONAL AND PREDICTIVE DATA				1			
FUNCTIONAL DATA					1		
SEGREGATION DATA				1			
DE NOVO DATA							
ALLELIC DATA							
OTHER DATABASE				1			
OTHER DATA							

ClinGen Pathogenicity Calculator: Use case 2

Step-by-step instructions for the interactive exercise to be presented at the ClinGen Workshop at ASHG 2015.

Workshop page: http://calculator.clinicalgenome.org/ashg-2015
Variant: NM_000169.2:c.639+919G>A
Gene: GLA/Fabry Disease

Pathogenicity Calculator and ACMG guidelines for variant interpretation

Previous presentation (Heidi Rehm) reviewed ACMG guidelines.

ACMG guidelines provide:
- Systematic categorization of evidence types and their strength
- Rules for making conclusions about pathogenicity based on the evidence

Rule application may be a tedious, sometimes error-prone process that may be hard to track and document and may involve personnel at various competence levels

Pathogenicity Calculator eliminates error in rule application and provides tracking of evidence used to reach specific conclusions.

ACMG guidelines provide categorization of evidence and explicit rules for reaching conclusions about pathogenicity

ACMG Evidence Tags

BS1, BS2, BS3, BS4,
BP4, BP1, BP7, BP3, BP2, BP6, BP5,
PP1, PP2, PP3, PP4, PP5
PM2, PM5, PM4, PM1, PM6, PM3,
PS1, PS2, PS3, PS4,
PVS1

Upgrading/Downgrading Strength (Examples)

BS1-Supporting, BS2-Supporting
PP1-Strong, PS1-Supporting

Pathogenicity Evidence grid

Five cells contain one piece of evidence each in favor of pathogenicity.

One may be inclined to assert the variant is pathogenic.

However, the strongest assertion that can be reached using ACMG rules is "Likely Pathogenic".

Thus, application of rule-based reasoning is important when interpreting evidence.

Pathogenicity Evidence

Phenotype: Colon cancer	Benign			Pathogenic			
	Supporting	Strong	Stand Alone	Supporting	Moderate	Strong	Very Strong
POPULATION DATA					1		
COMPUTATIONAL AND PREDICTIVE DATA				1			
FUNCTIONAL DATA				1			
SEGREGATION DATA							
DE NOVO DATA							
ALLELIC DATA							
OTHER DATABASE				1			
OTHER DATA				1			

Overview of Use Case 2

Allele: NM_000169.2:c.639+919G>A

Step 1: Identify Allele
Step 2: Launch the Calculator
Step 3: Create evidence document and input evidence
Step 4: Calculate conclusions and examine reasoning
Step 5: Retrieve stored evidence and conclusions

Allele: NM_000169.2:c.639+919G>A

Gene:GLA (alpha galactosidase)

Allele selected for curation in clinical sequencing and exploratory research (CSER)

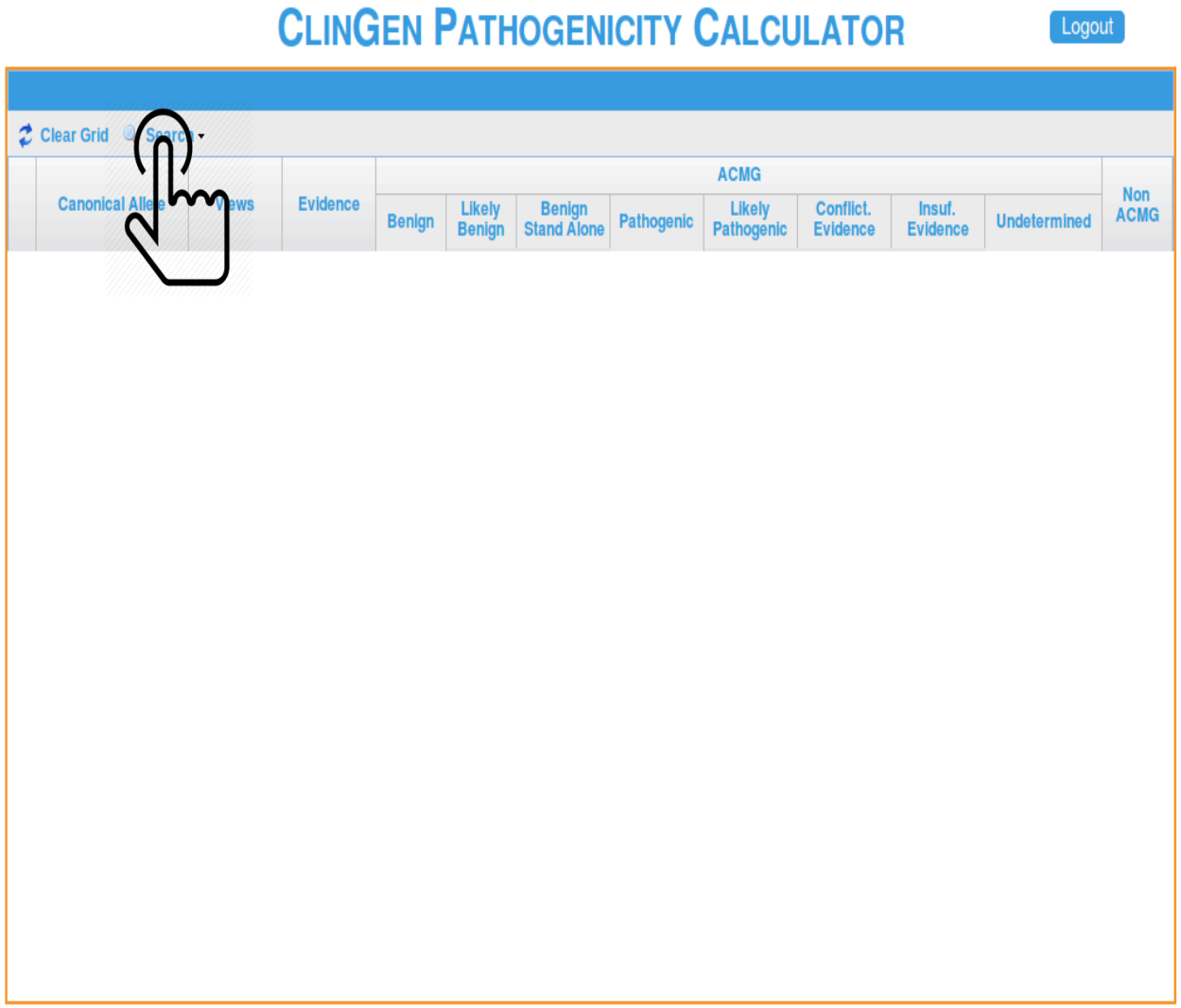
Three groups curated the variant with PP1-Moderate,PS3, PS4,PVS1, PM4,PP1,PP5, BP4,PP3 tags, leading to 3 different conclusions per ACMG Guidelines:

Pathogenic, Likely Pathogenic, Uncertain Significance

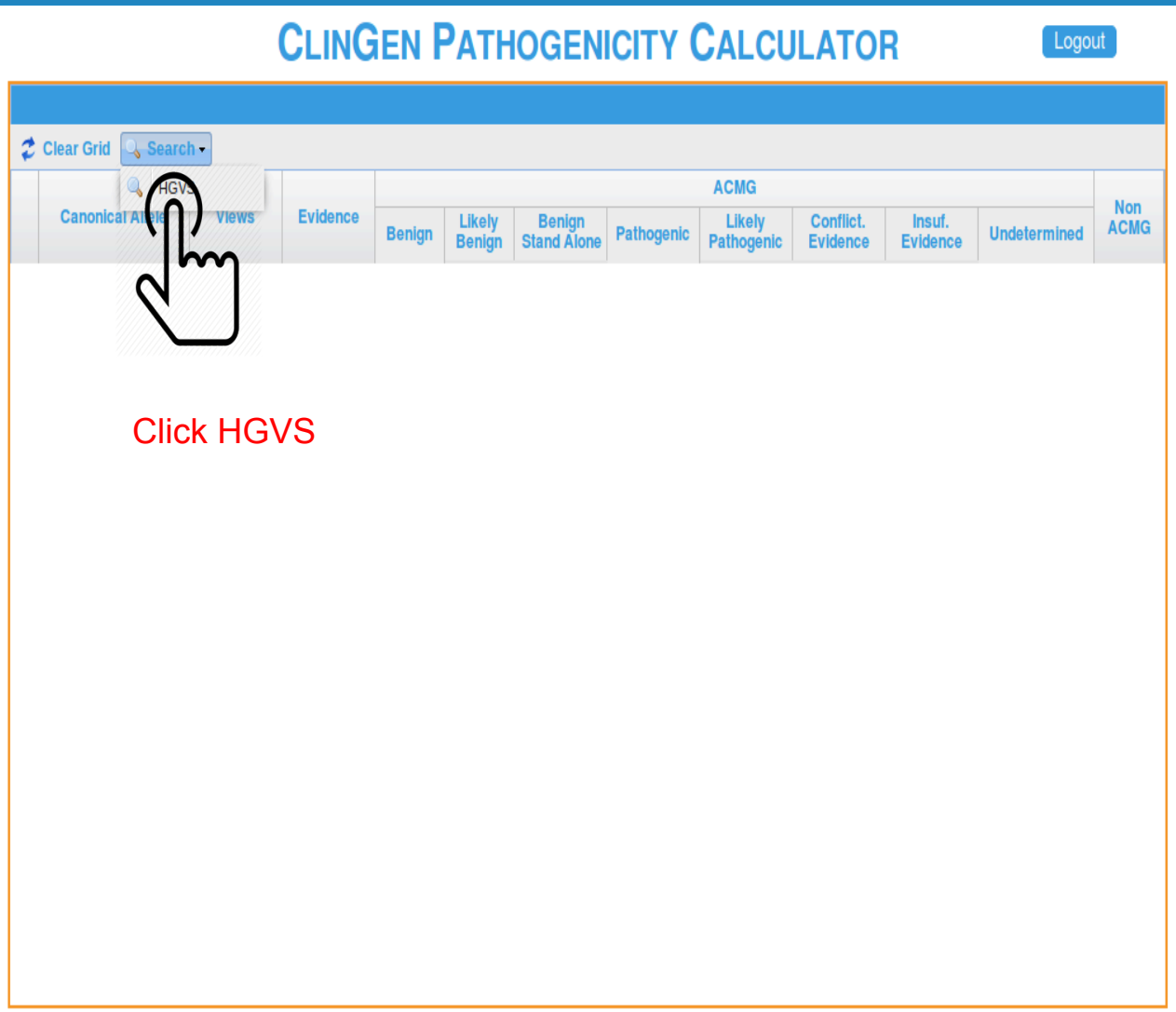
Consensus curation agreed on the following evidence tags for Fabry disease: PS4, PVS1-Strong, PS3, PP1

In the present use case, these four evidence tags will be used for this allele to calculate conclusion based on ACMG guidelines

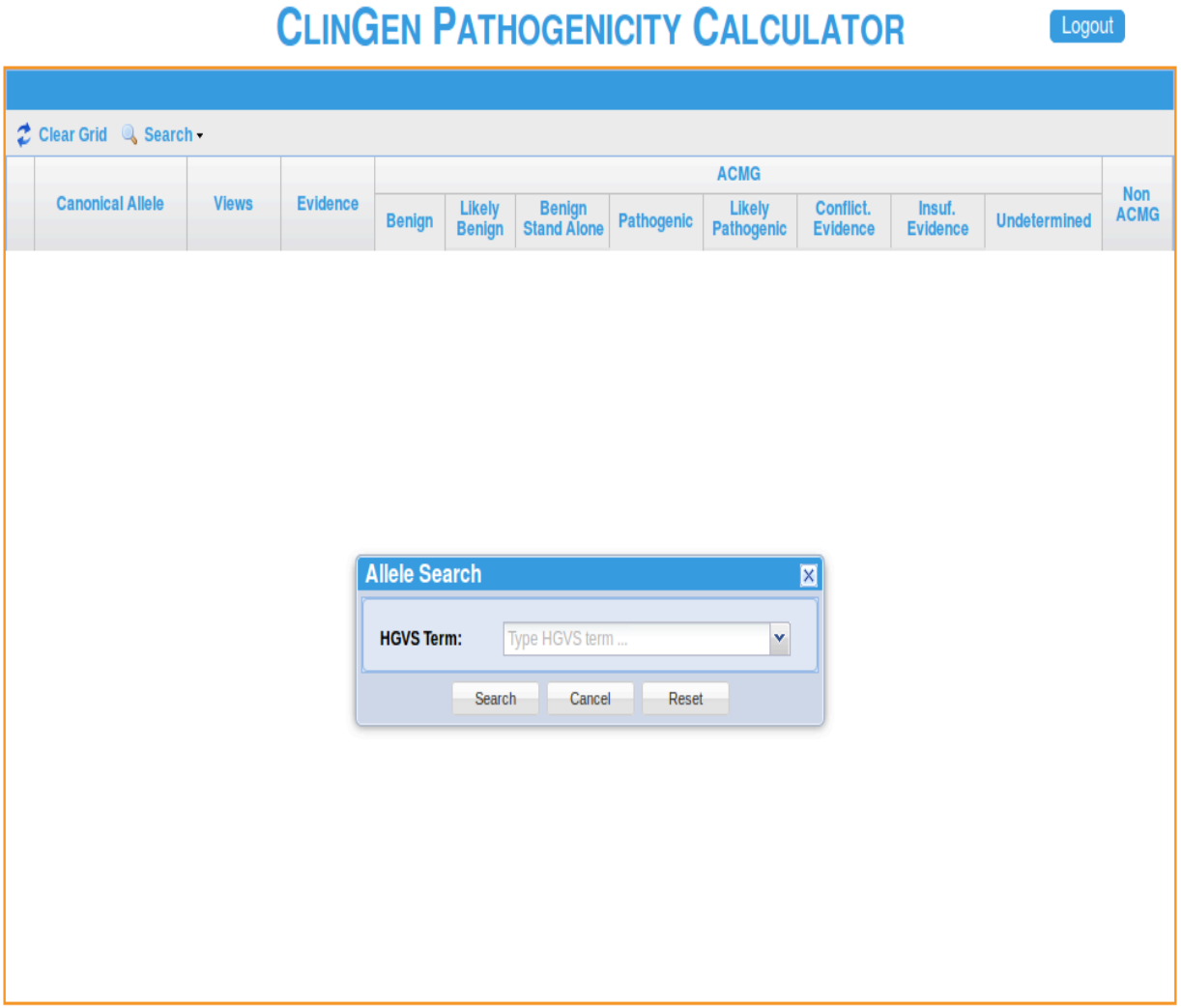
Step 1: Identify allele: Click on search



Step 1: Identify allele: Click on HGVS

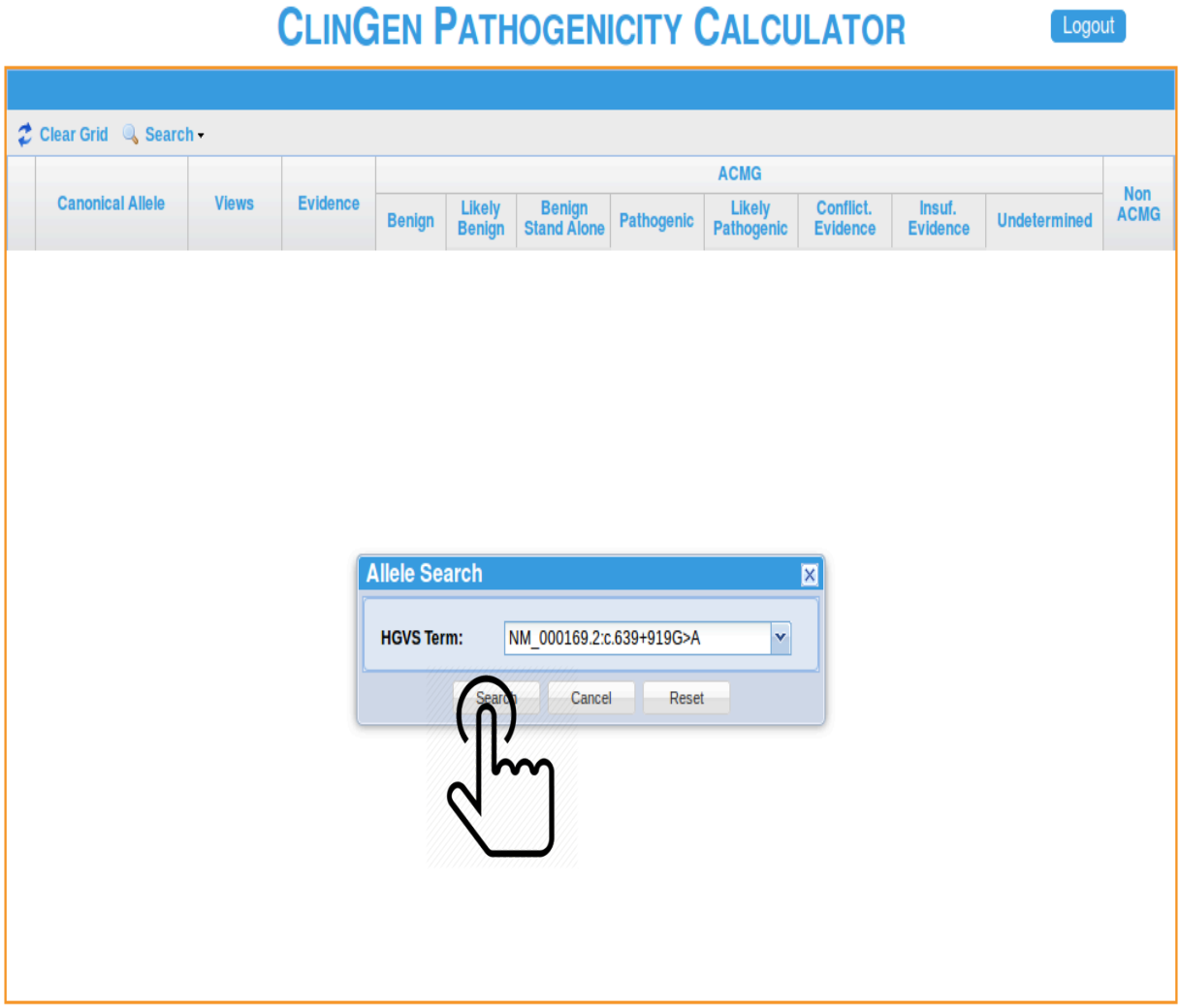


Step 1: Identify allele: The allele Search panel pops up

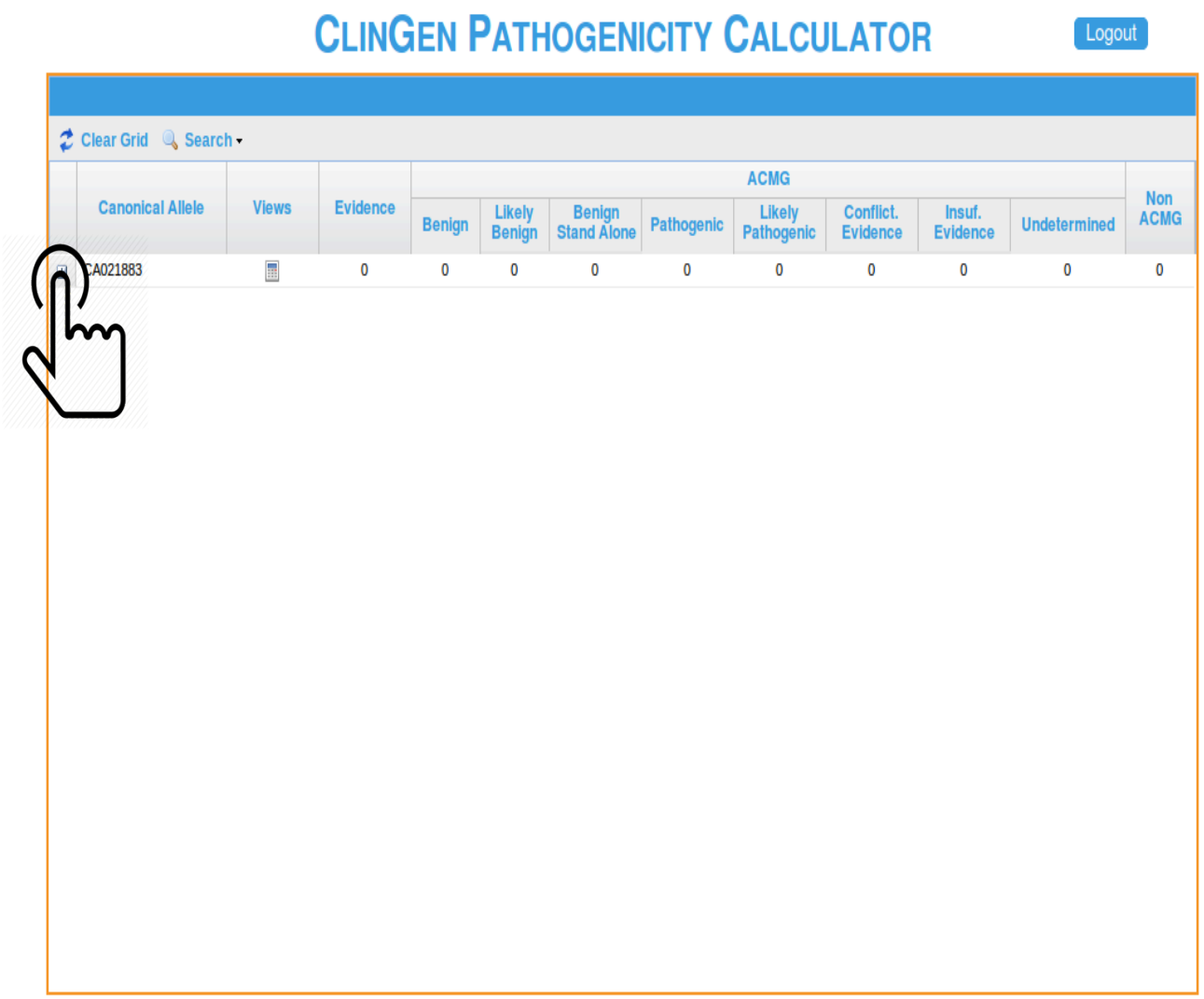


Step 1: Identify allele: The allele search panel pops up.

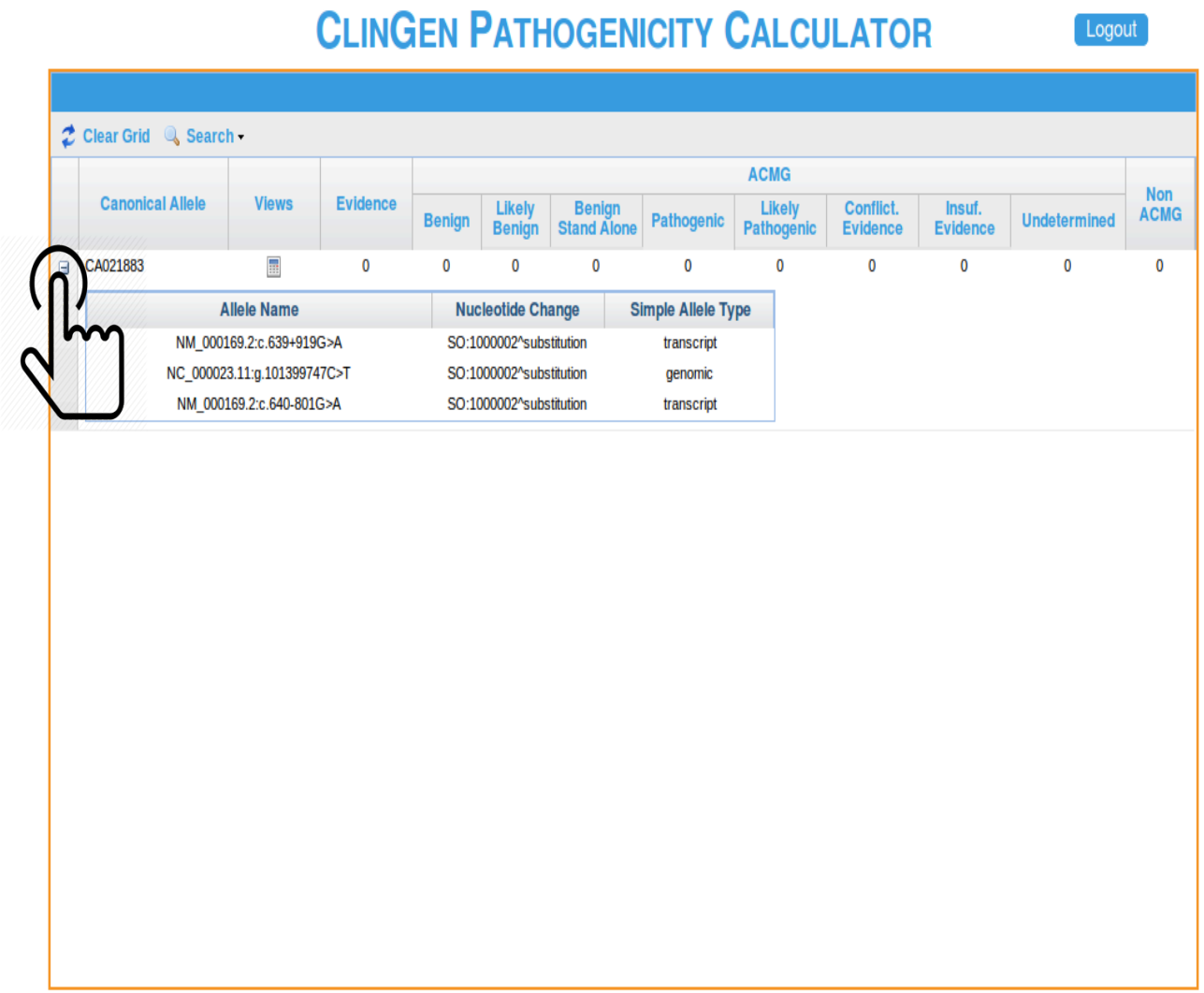
Search: **NM_000169.2:c.639+919G>A**



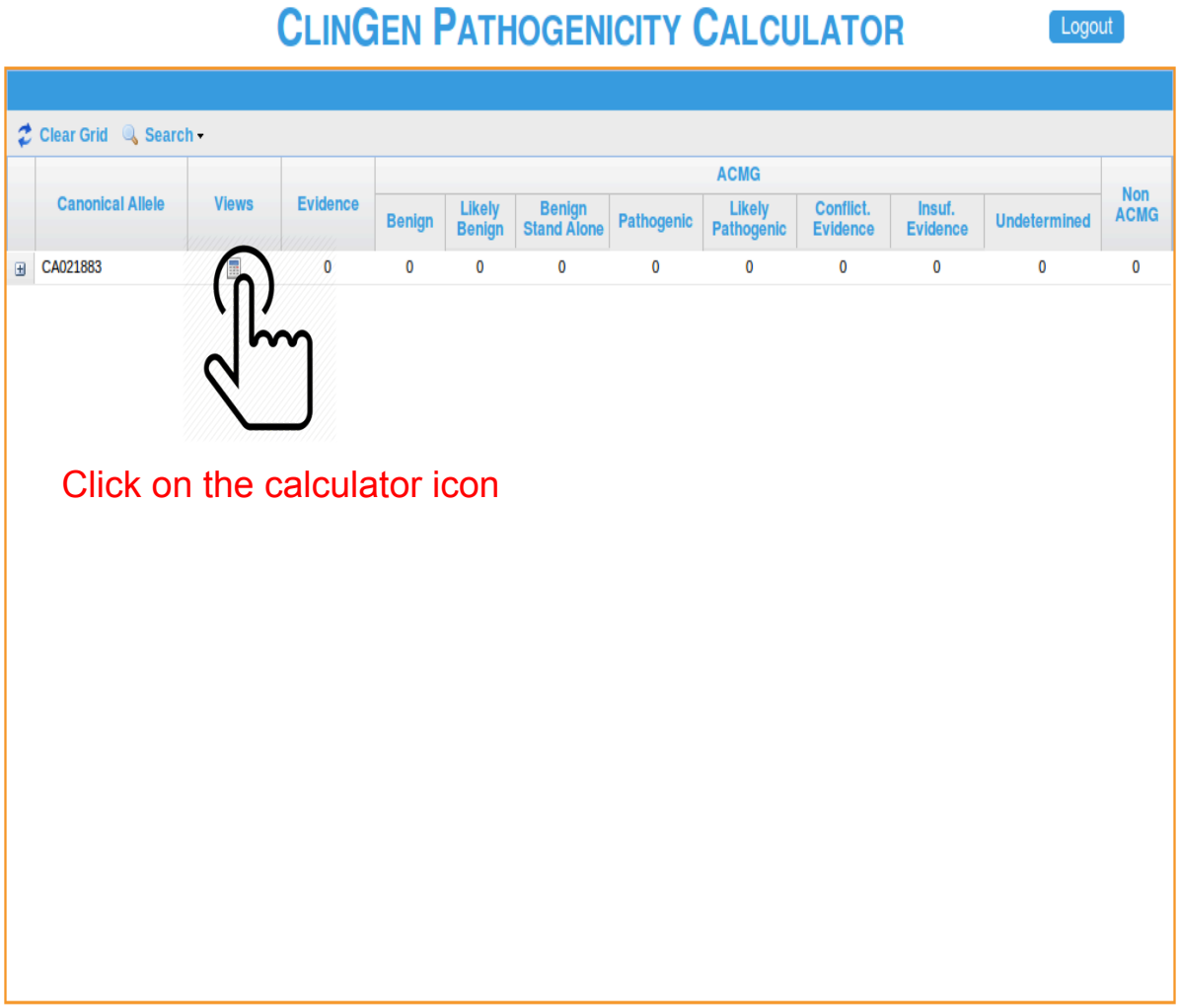
Step 1: Identify allele: View search results



Step 1: Identify allele: Inspect equivalent allele representations and confirm allele identity



Step 2: Launch the calculator

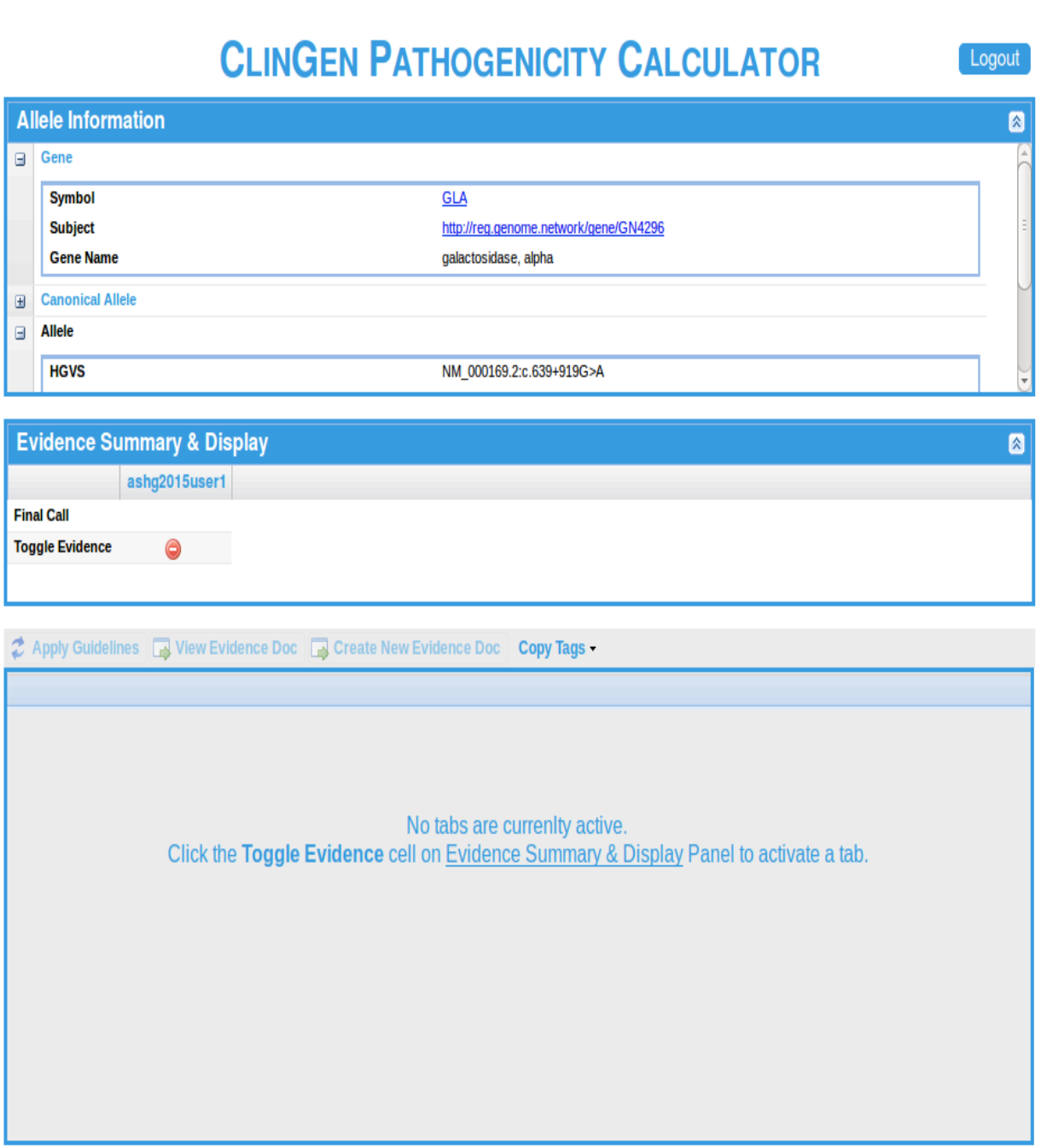


Learn more about gene/ allele

Step 2: Launch the calculator: Open the calculator tab

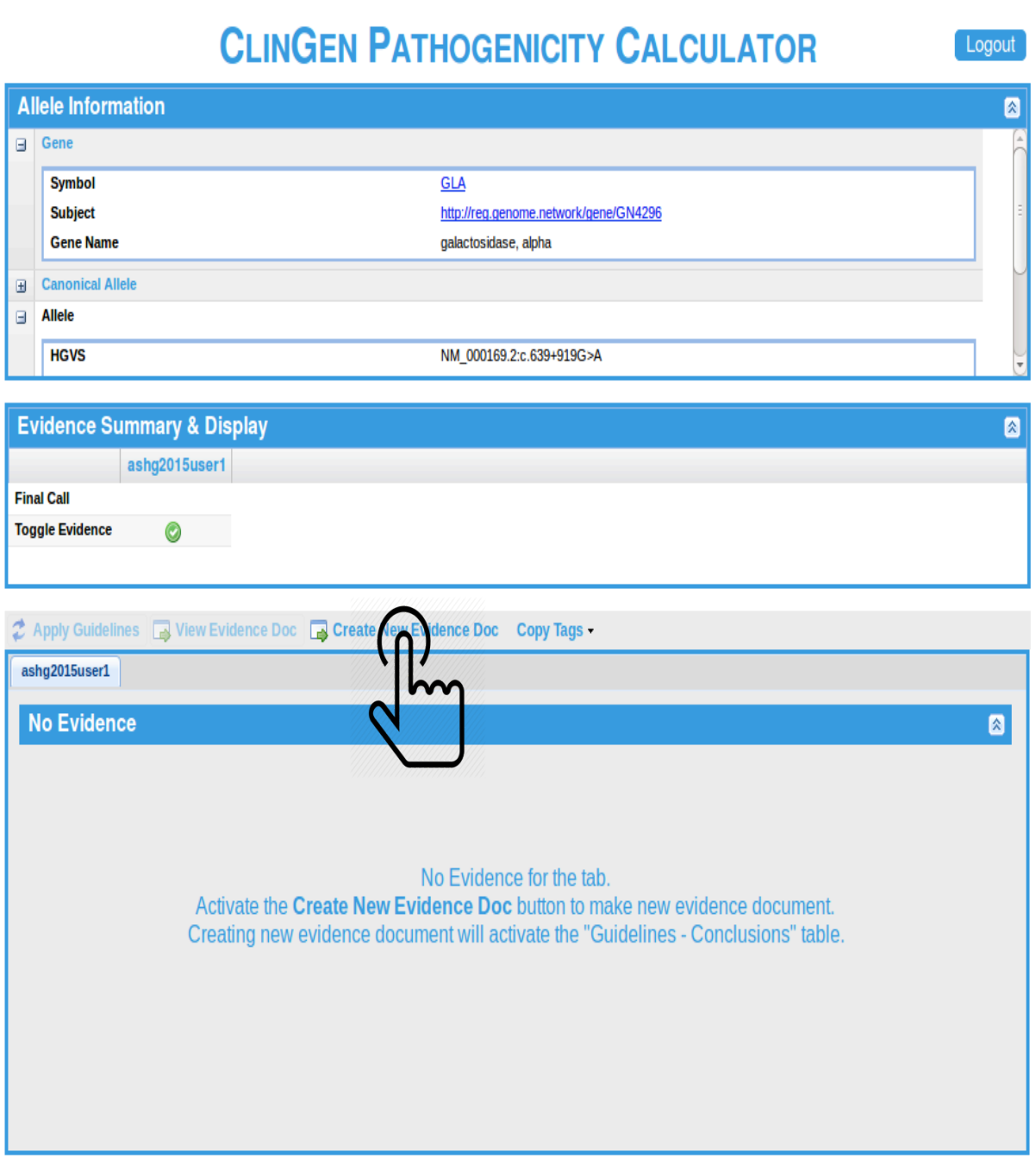
Because the evidence document is empty, the tab is not displayed

Click on the red circle (with “-” sign) in “Toggle Evidence” row



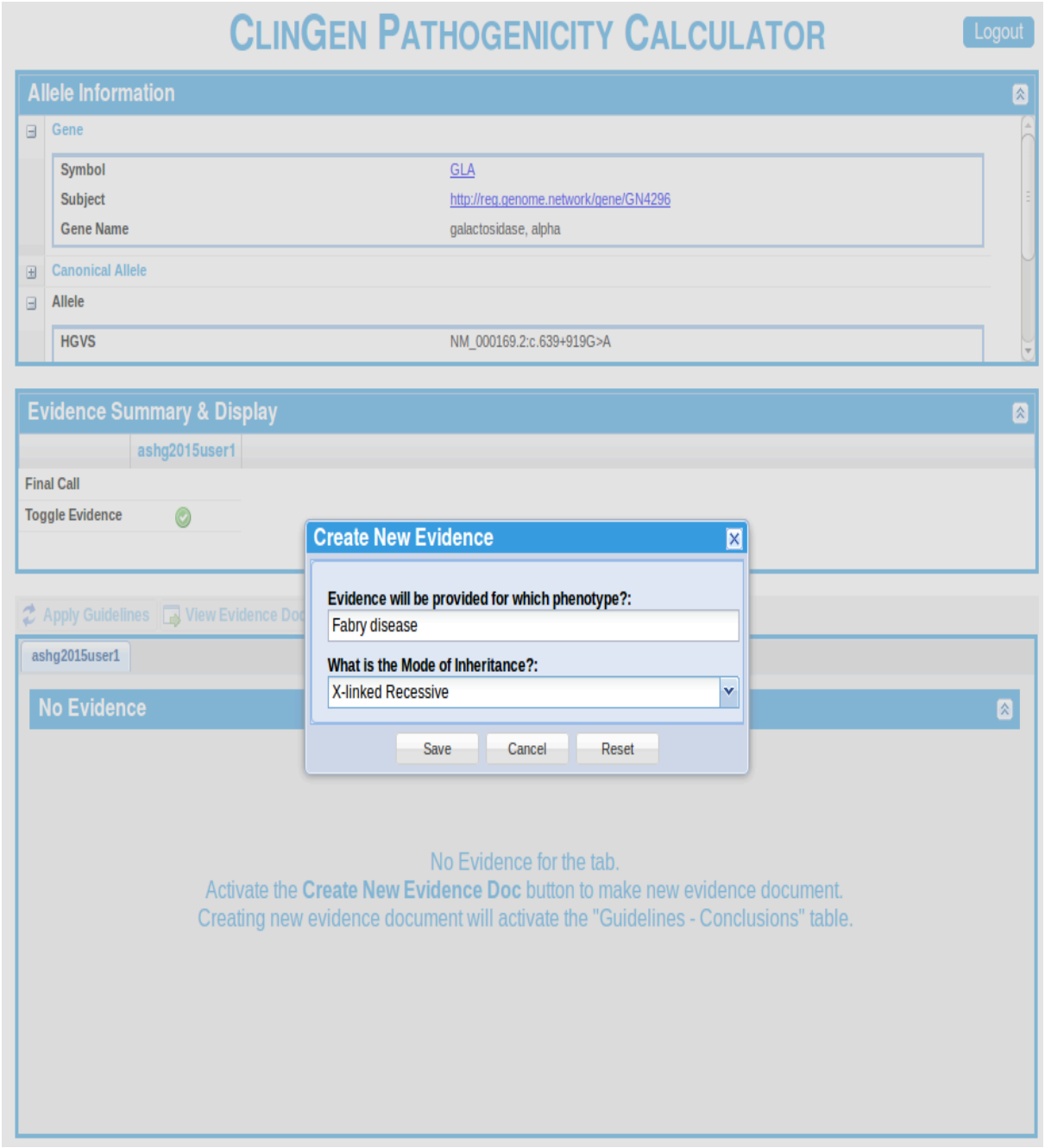
Step 3: Create evidence document and input evidence

The new evidence document that you will create now will be populated by evidence tags for this allele



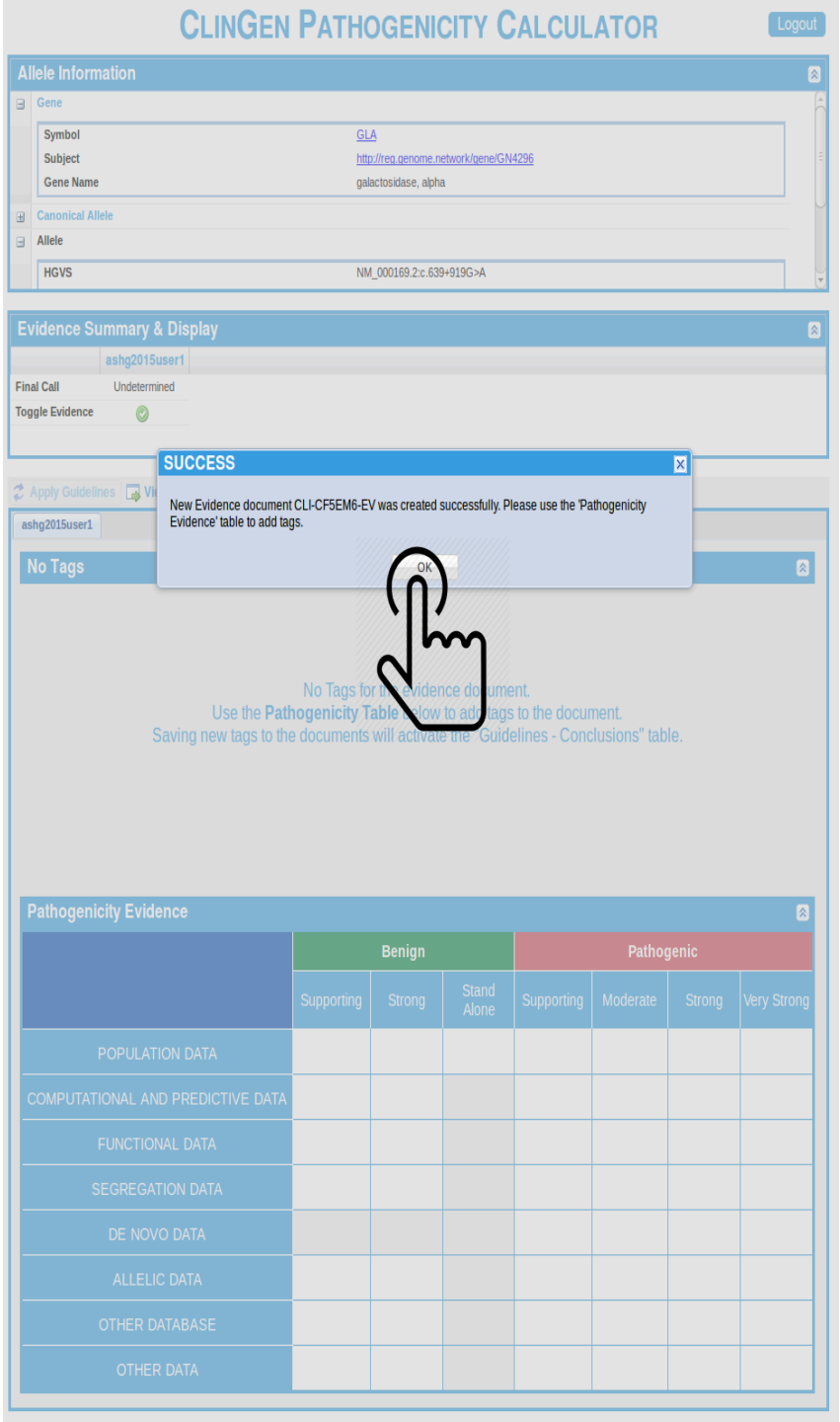
Step 3: Create evidence document and input evidence: Provide basic information

Provide information about condition and mode of inheritance

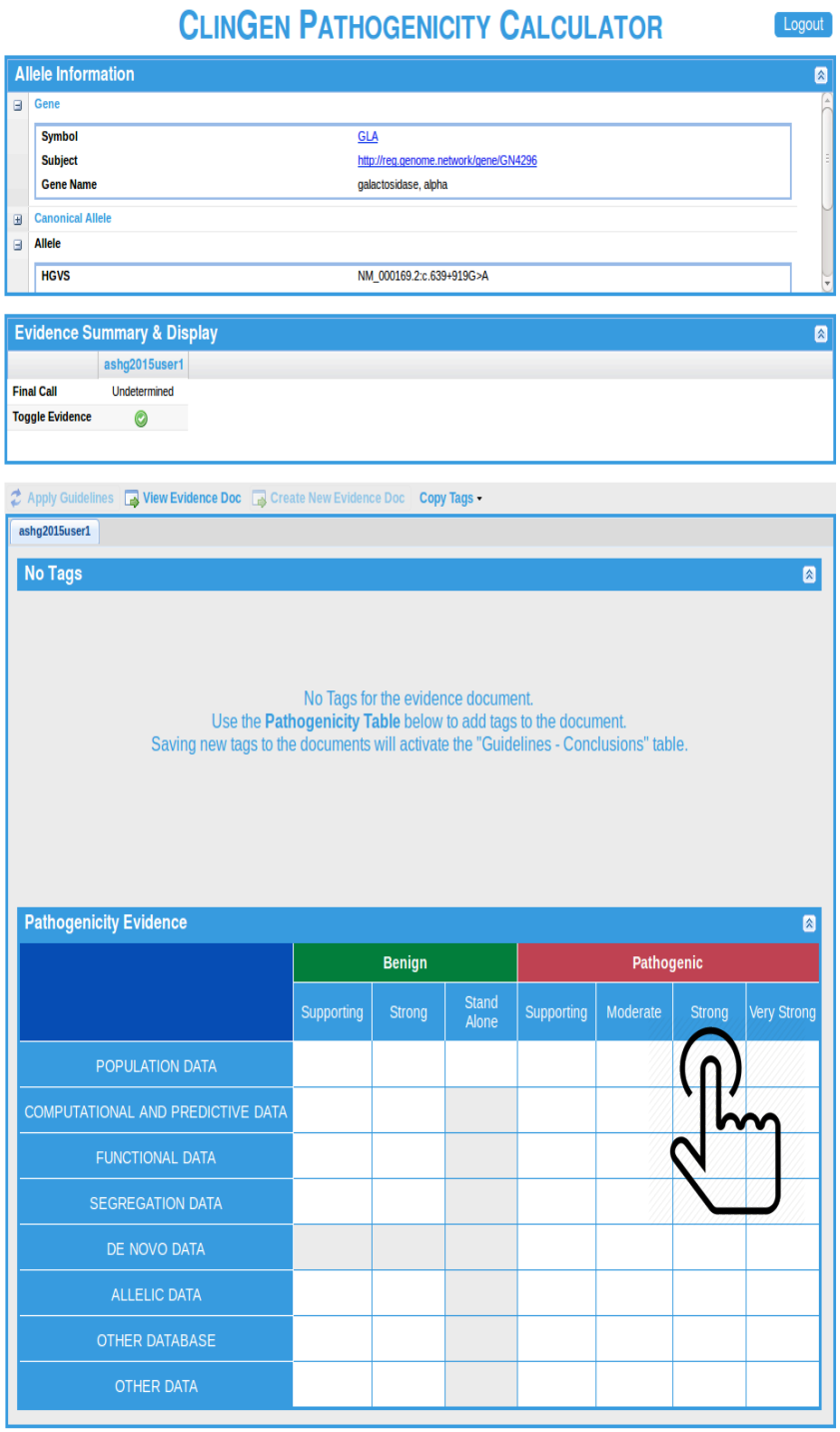


Step 3: Create evidence document and input evidence

Click OK to notification

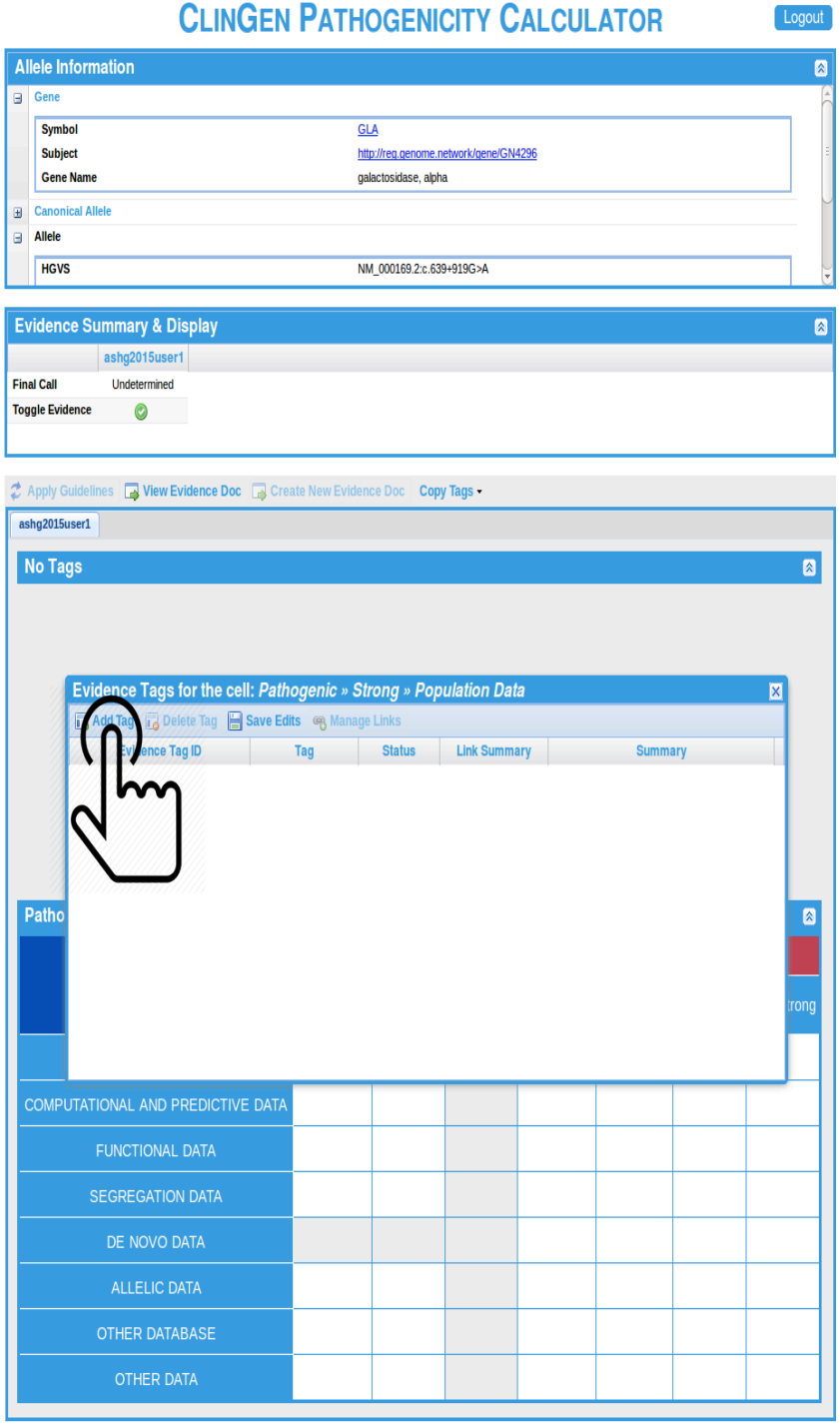


Step 3: Create evidence document and input evidence: Turn PS4 tag on



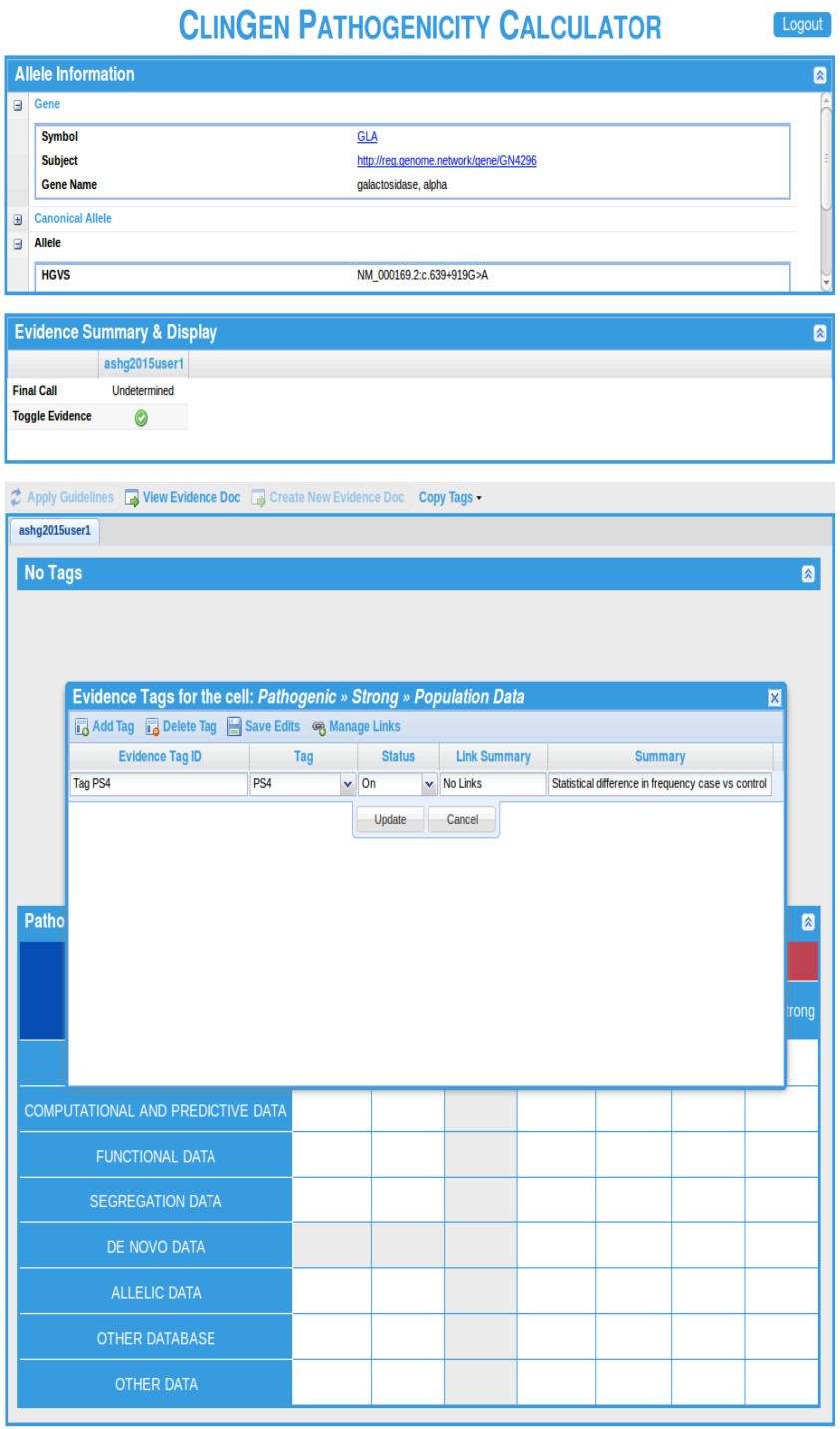
Step 3: Create evidence document and input evidence: Turn PS4 tag on

Click Add Tag

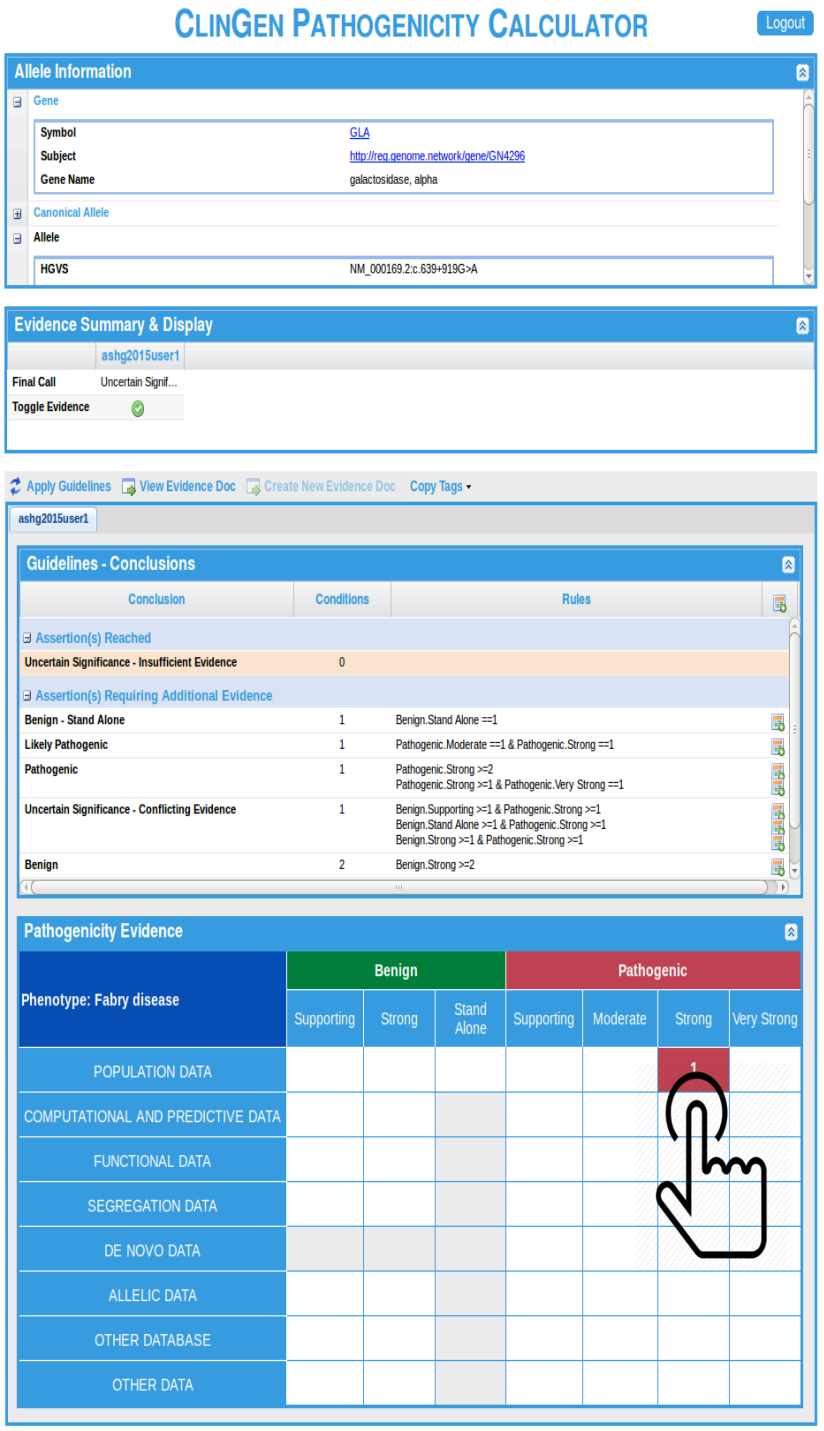


Step 3: Create evidence document and input evidence: Turn PS4 tag on

1. Add “Tag PS4” in “Evidence Tag ID” column
   This must be any unique string of characters
1. Select one of the tags from the pull-down menu
2. Optional text explaining why the tag is turned on
   This text may help remind you why you turned the tag on when you revisit this allele in the future
1. Press the Update button
2. Press the Save Edits button in the menu

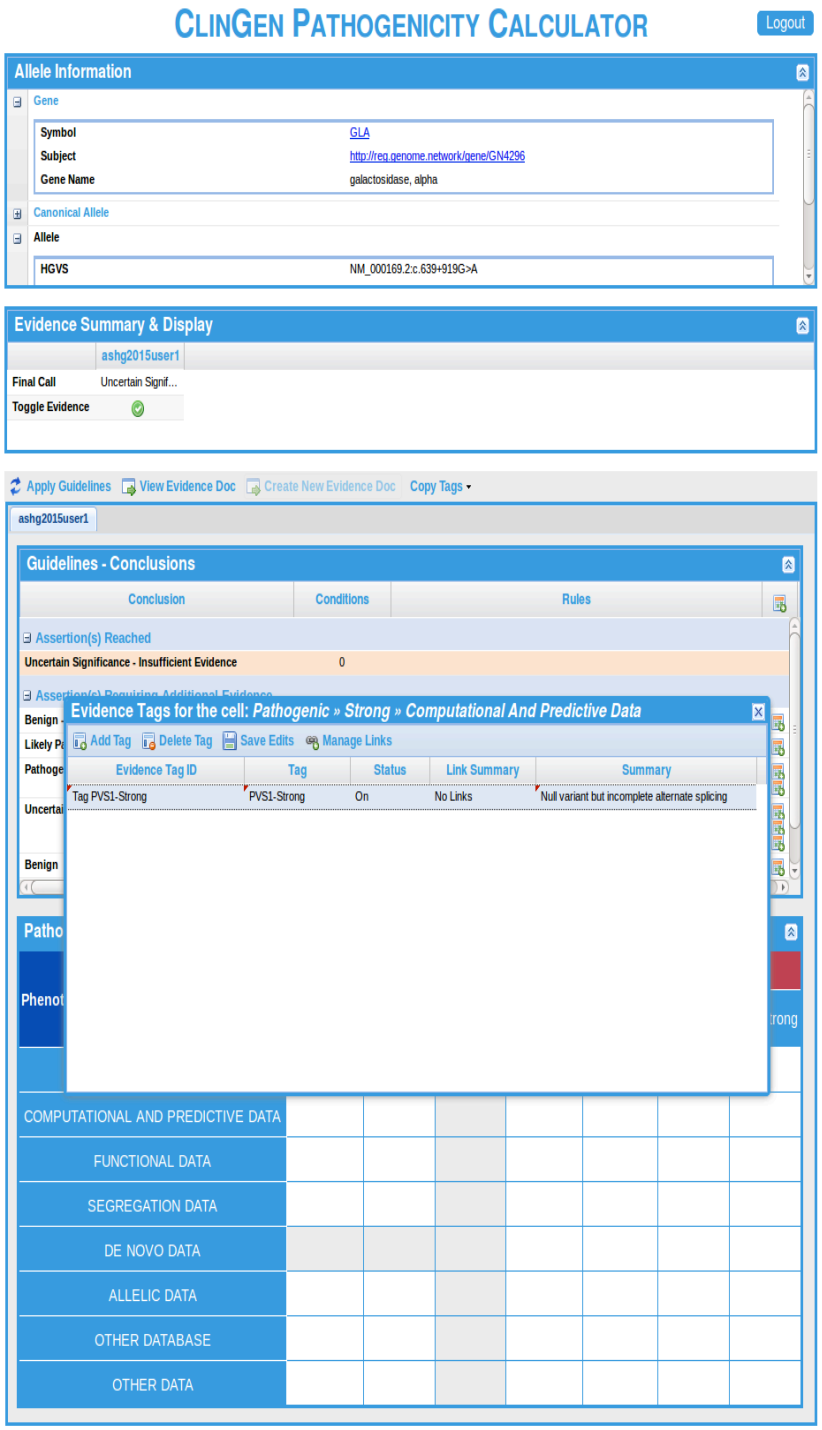


Step 3: Create evidence document and input evidence: Turn PVS1-Strong tag ON

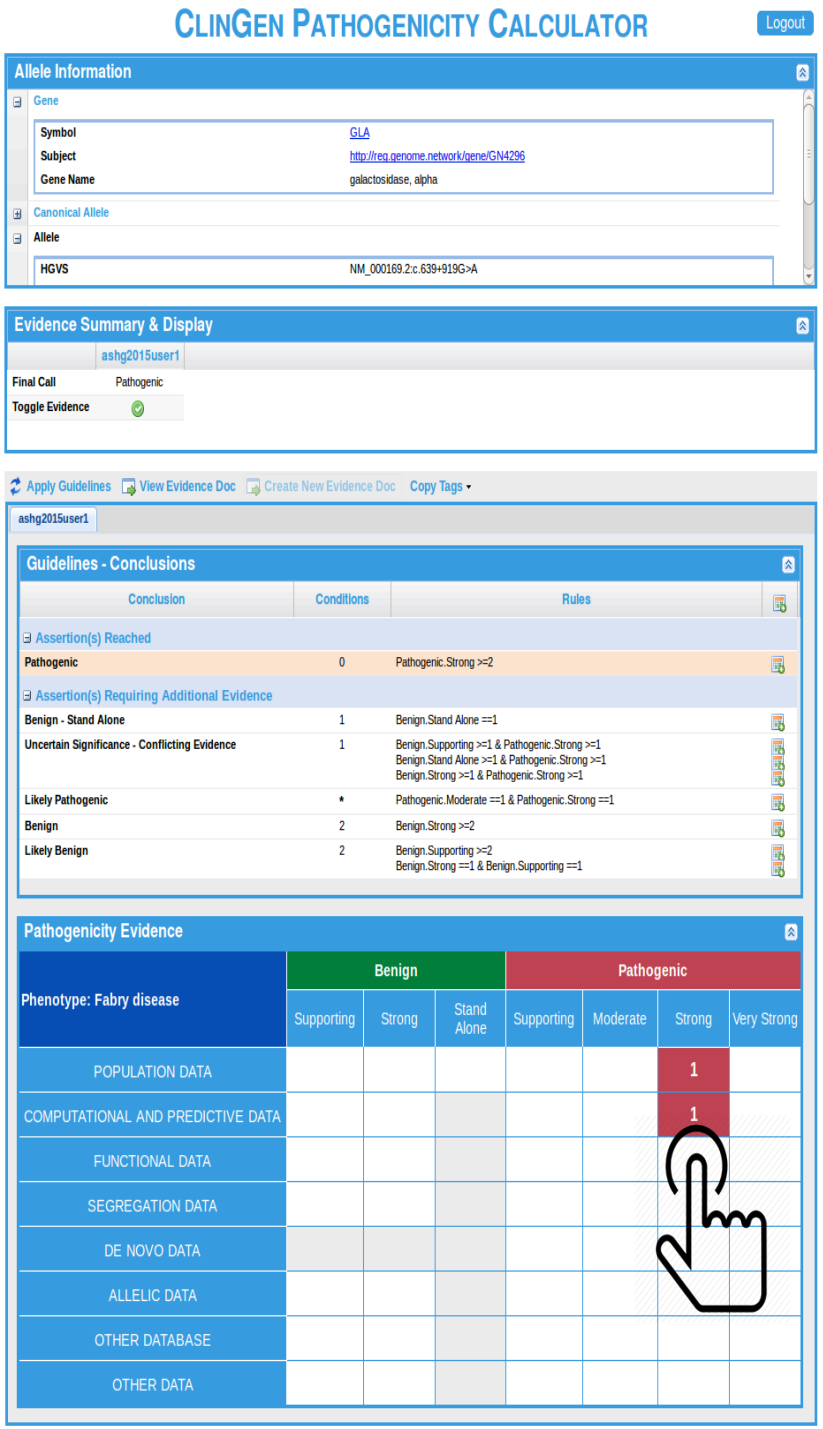


Step 3: Create evidence document and input evidence: Turn PVS1-Strong tag ON

See slide #21 for details

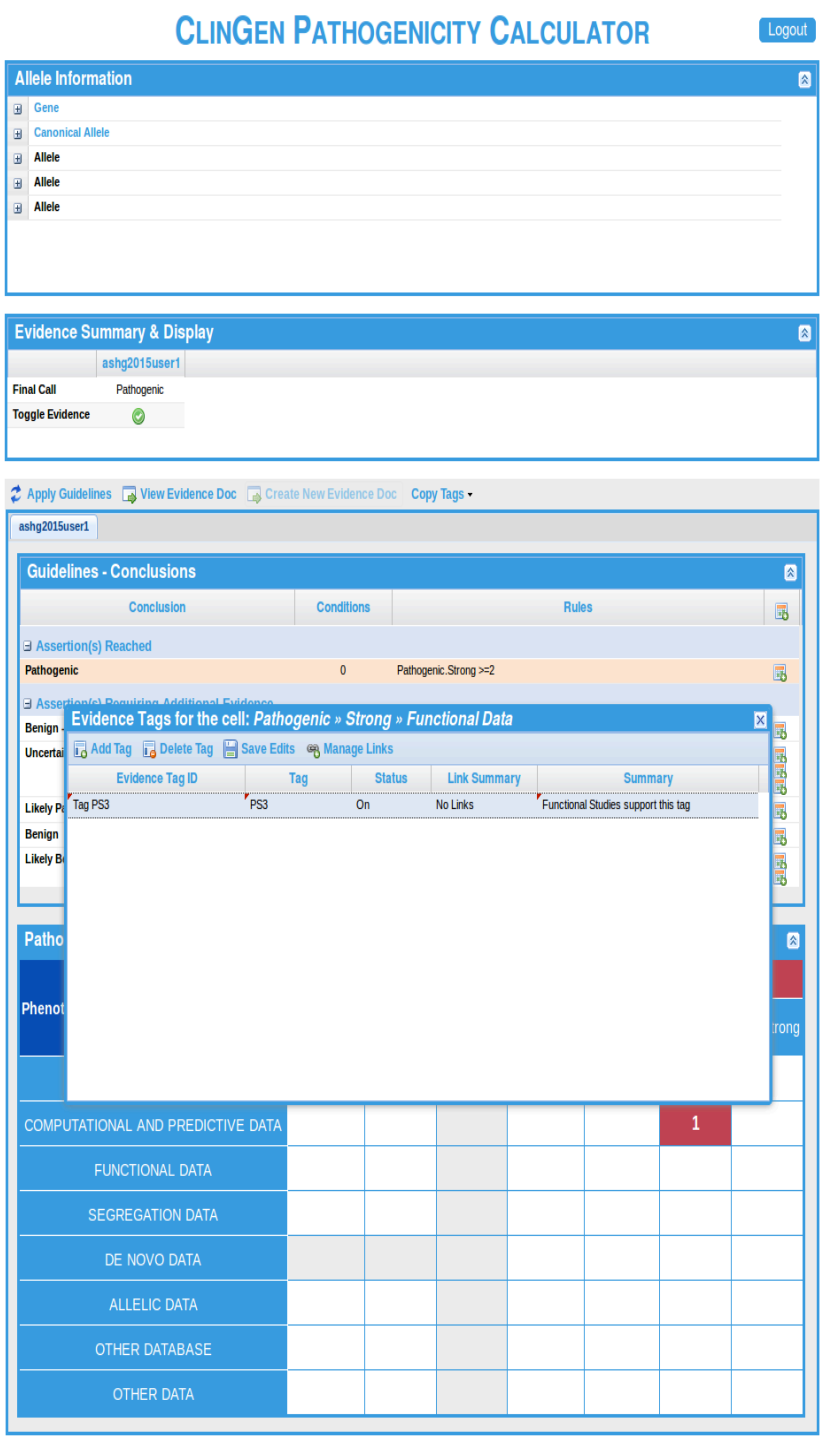


Step 3: Create evidence document and input evidence: Turn PS3 tag ON

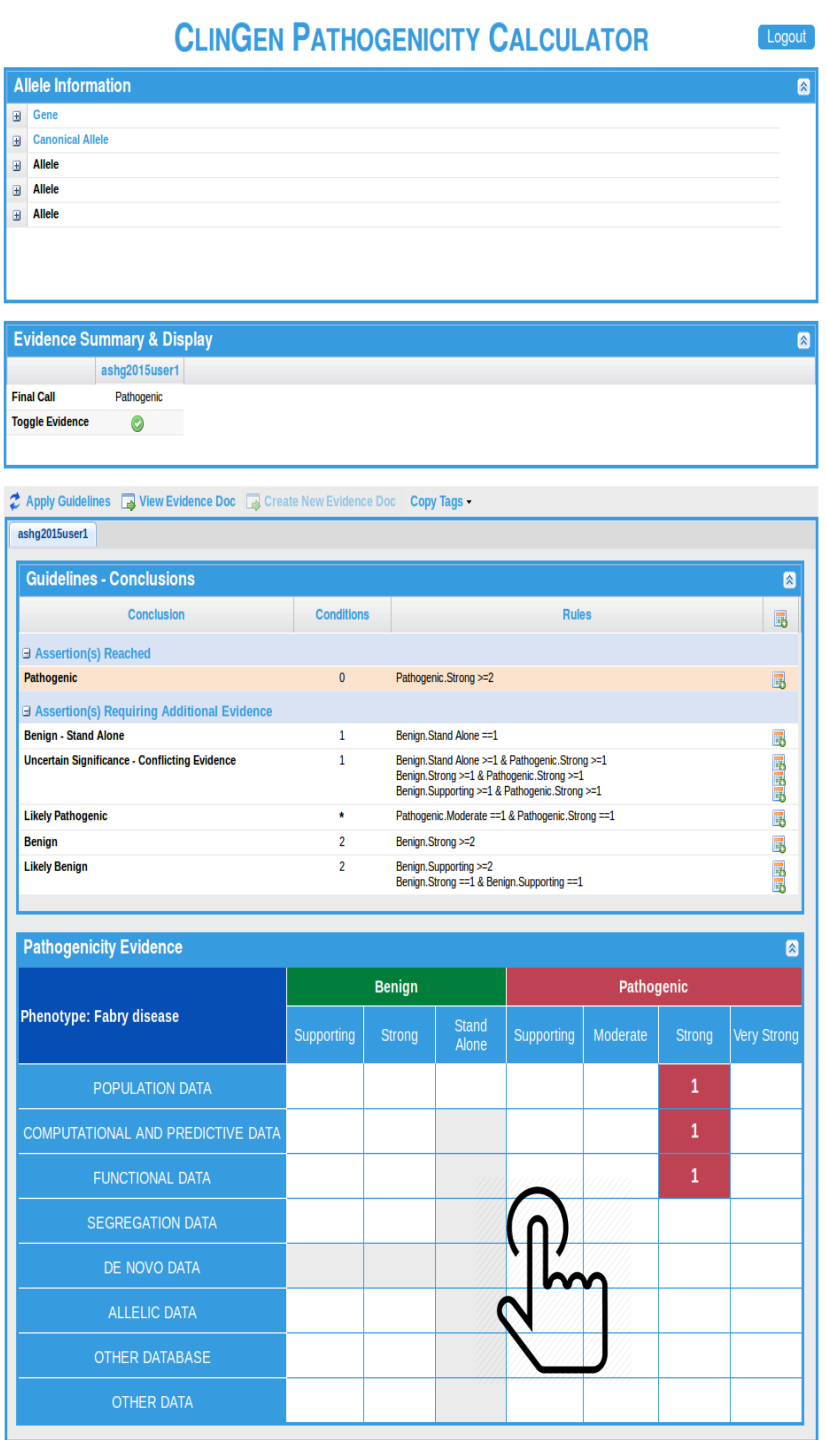


Step 3: Create evidence document and input evidence: Turn PS3 tag ON

See slide #21 for details

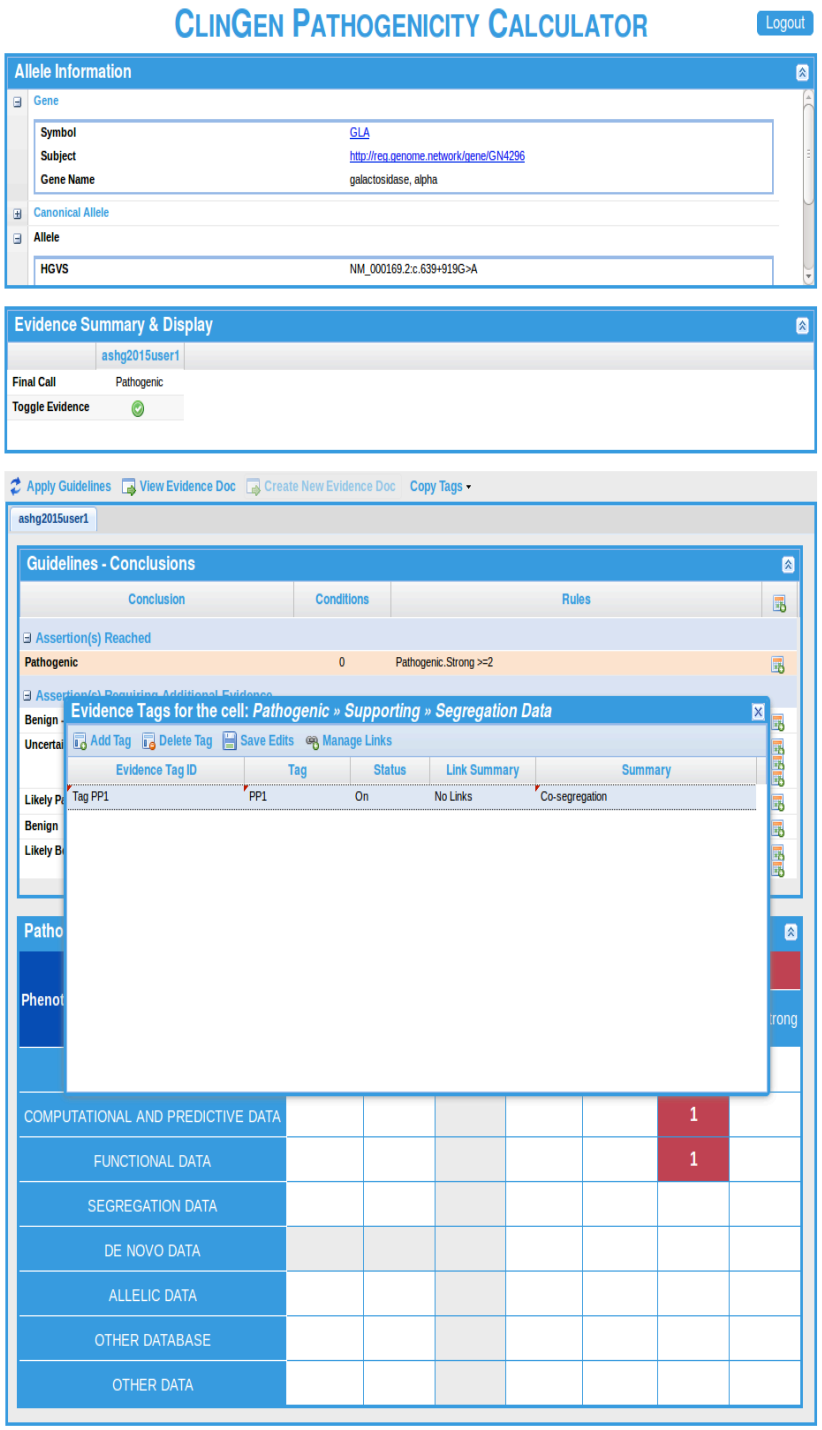


Step 3: Create evidence document and input evidence: Turn PS3 tag ON



Step 3: Create evidence document and input evidence: Turn PS3 tag ON

See slide #21 for details



Conclusion and Reasoning

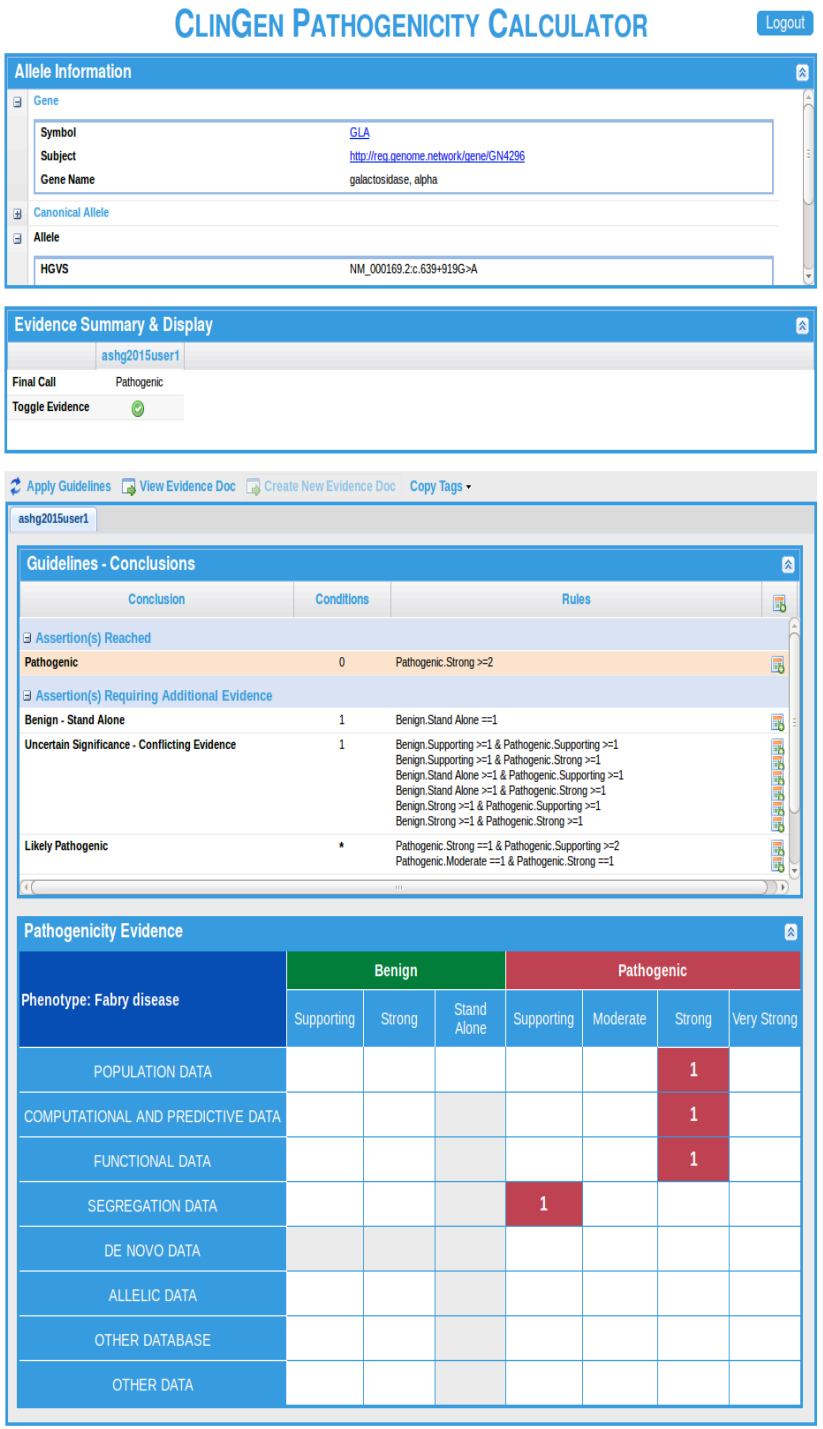
The conclusion reached is “Pathogenic”.

The rule that is satisfied is highlighted next to the conclusion.

The rules that are not satisfied are also listed below but are not highlighted.

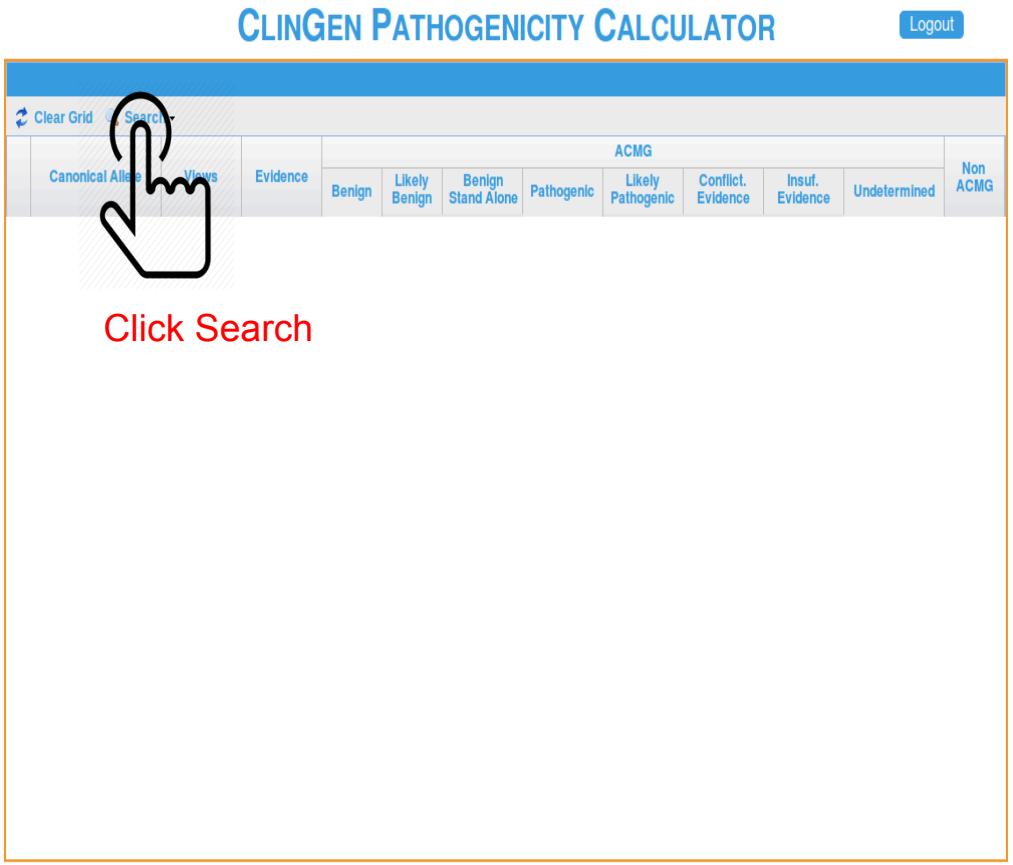
For each rule that is not satisfied, the number of missing evidence items is listed.

By clicking on the rule that is not satisfied, missing evidence items (grid columns) are highlighted, helping identify evidence tags that may lead to a conclusion.

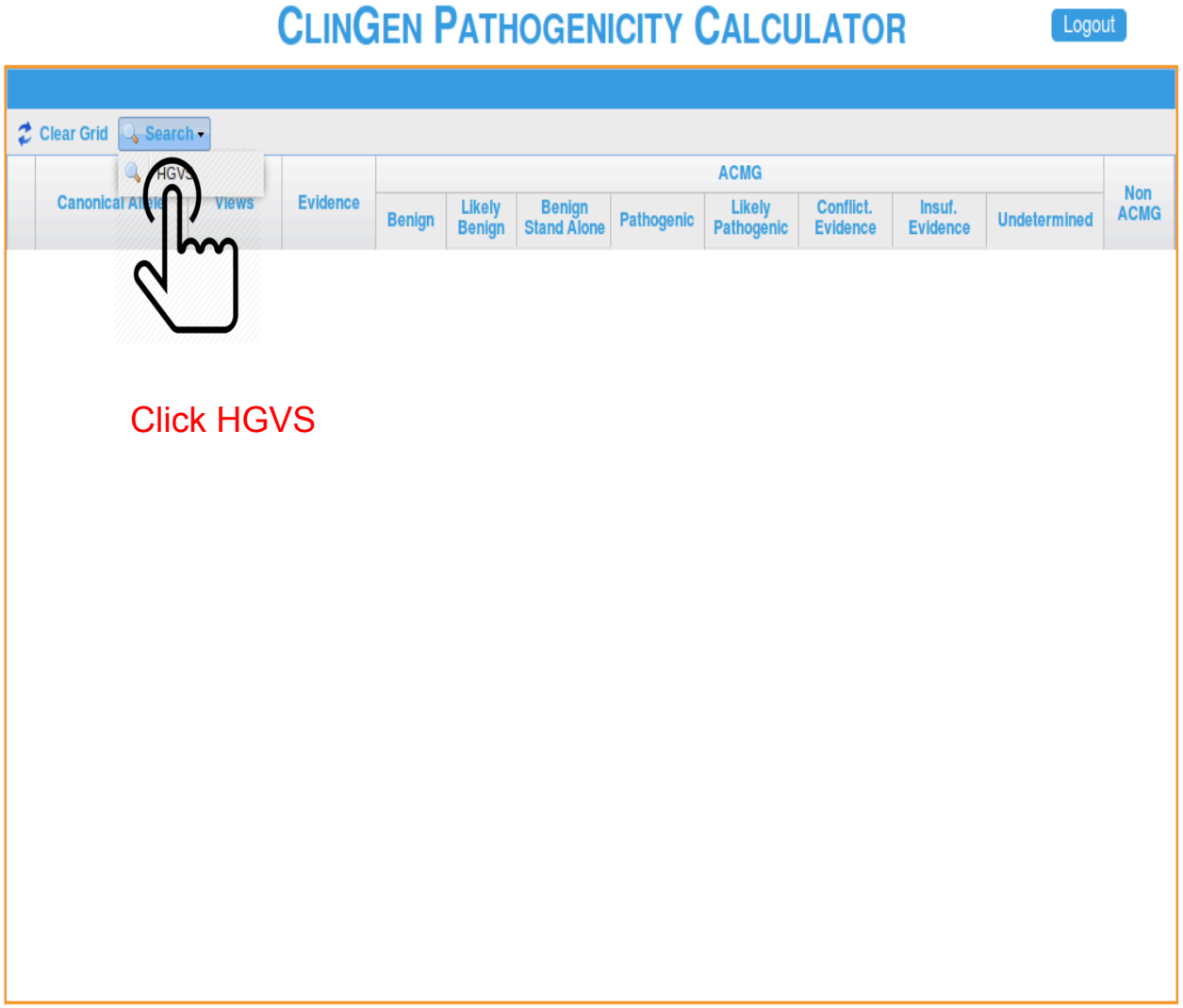


Step 5: Retrieve stored evidence and conclusions: Activate HGVS based search

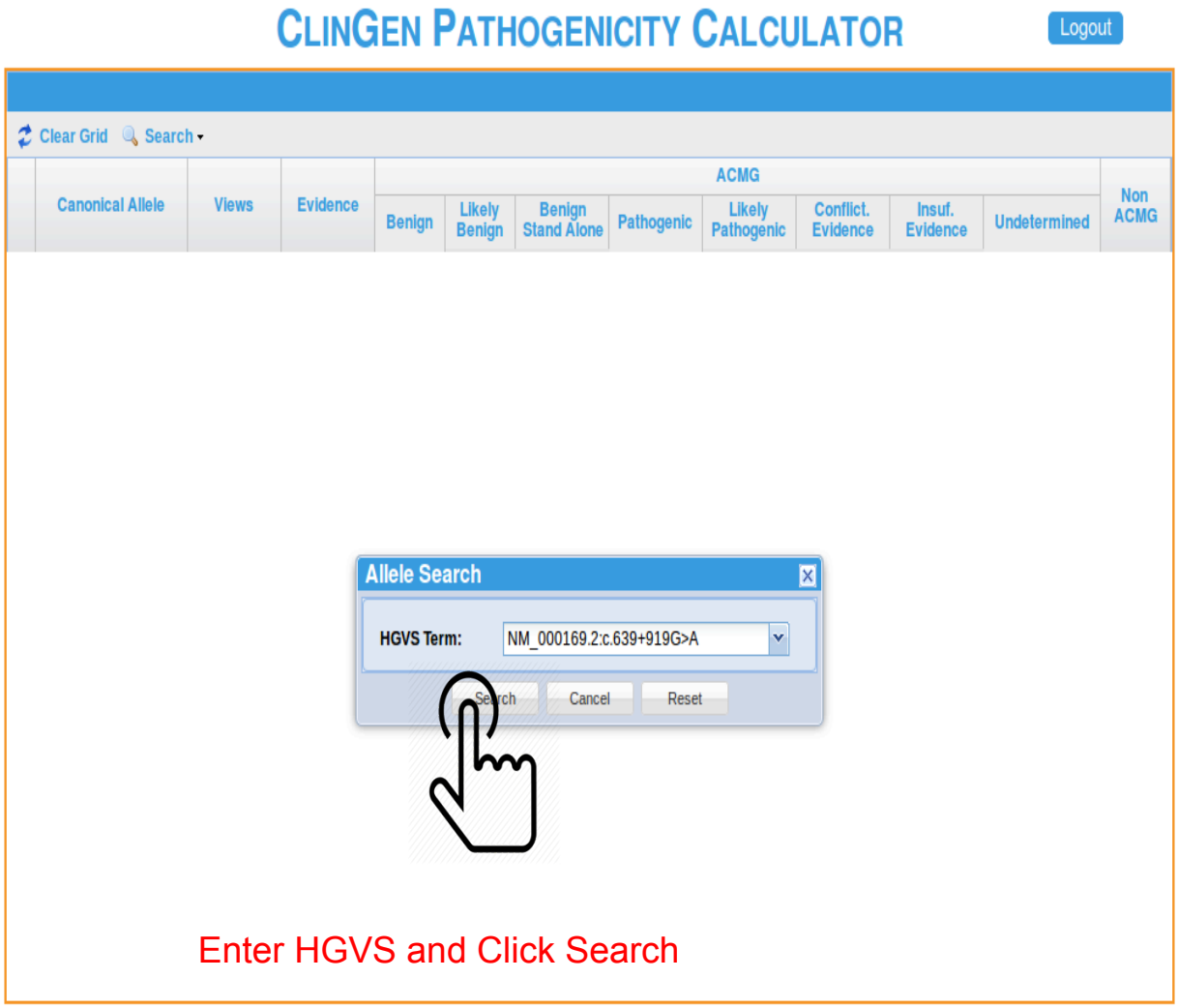
Visit: Perform the HGVS search for the same allele:
calculator.clinicalgenome.org/java-bin/clingenV2.0.jsp



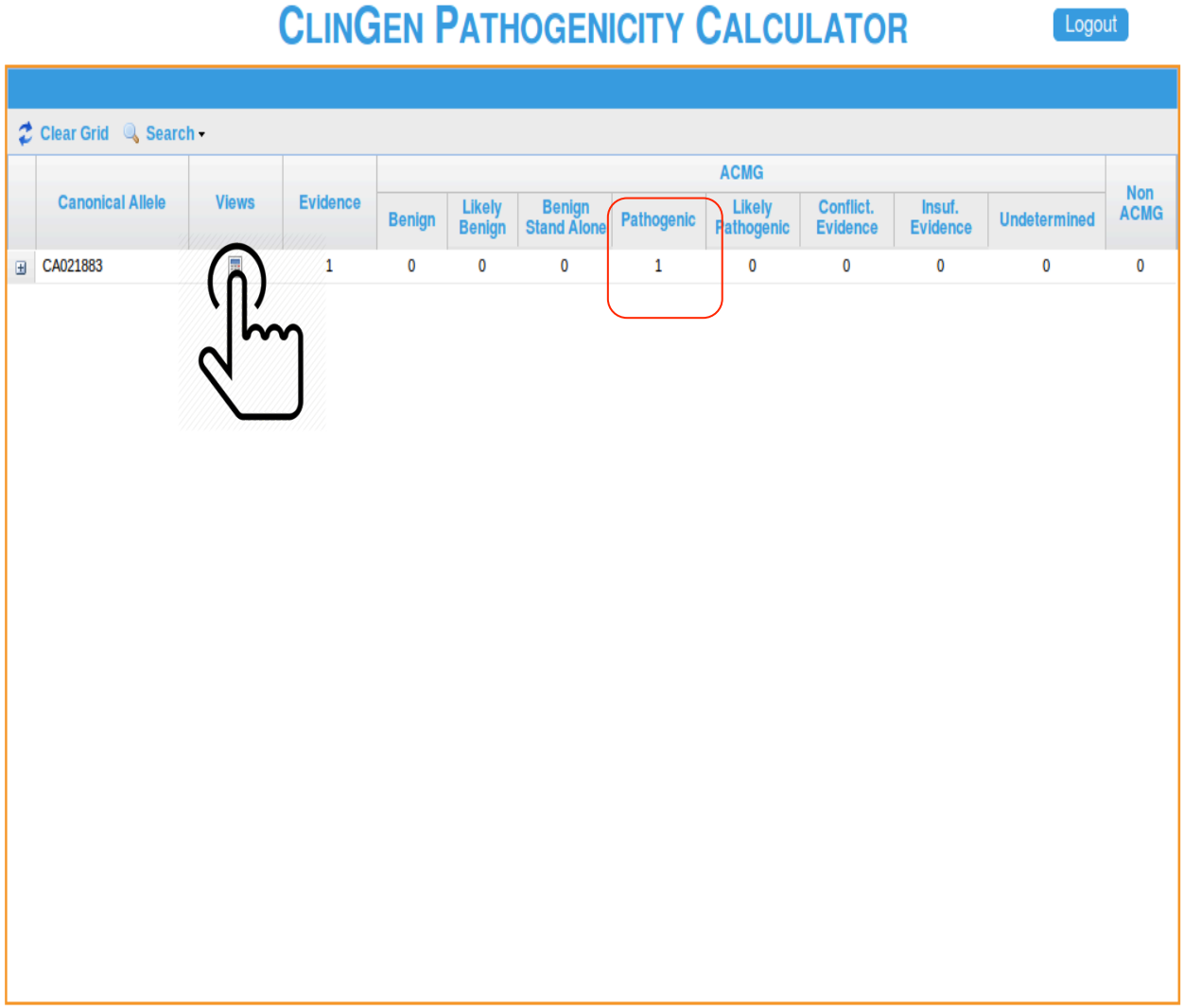
Step 5: Retrieve stored evidence and conclusions: Activate HGVS based search



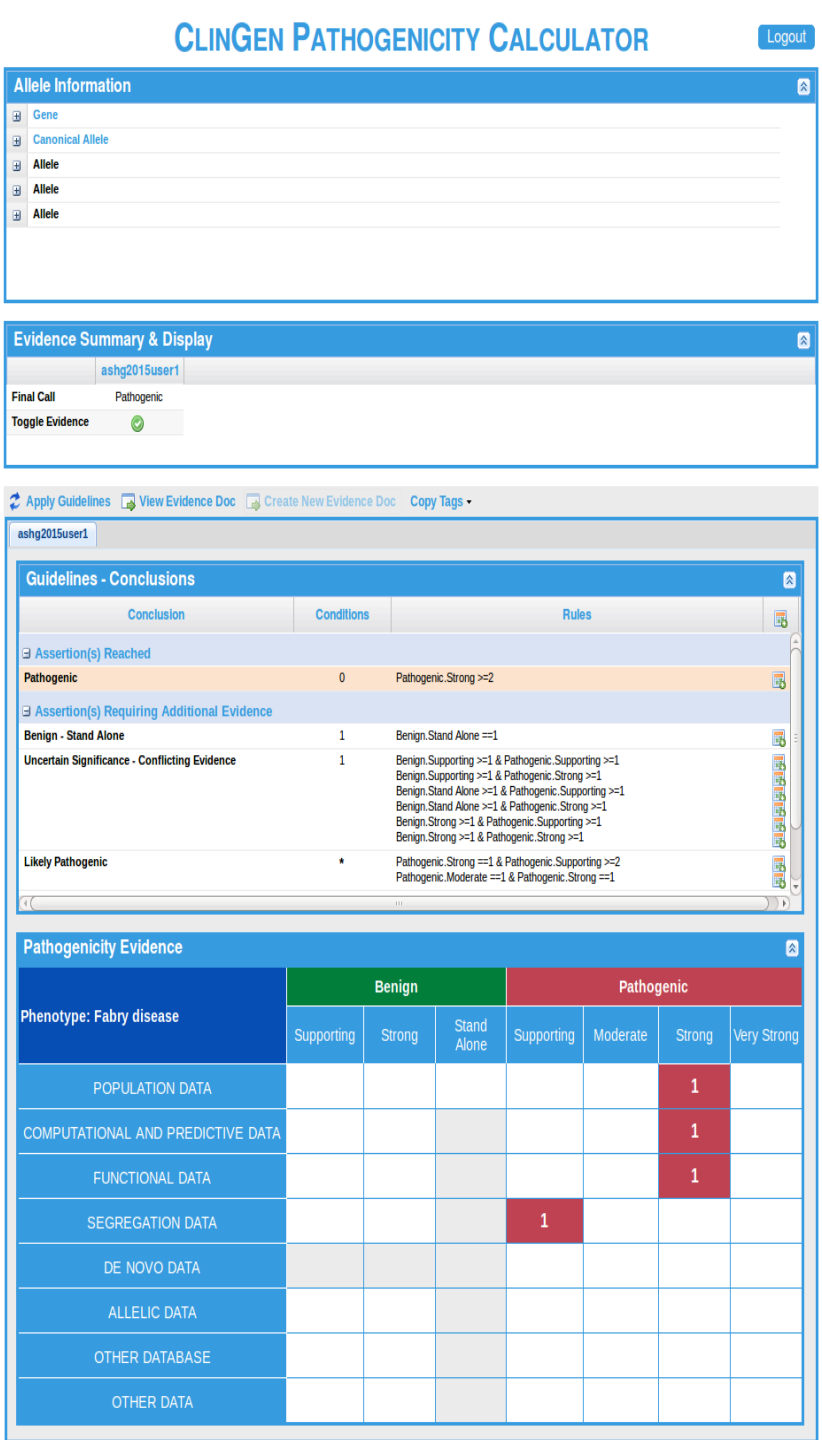
Step 5: Retrieve stored evidence and conclusions: Search for NM_000169.2:c.639+919G>A



Step 5: Retrieve stored evidence and conclusions: Launch the calculator to view evidence and conclusion



Step 5: Retrieve stored evidence and conclusion



Next: repeat exercise for the second variant

Search for:
NM_001369.2:c.7468_7488del

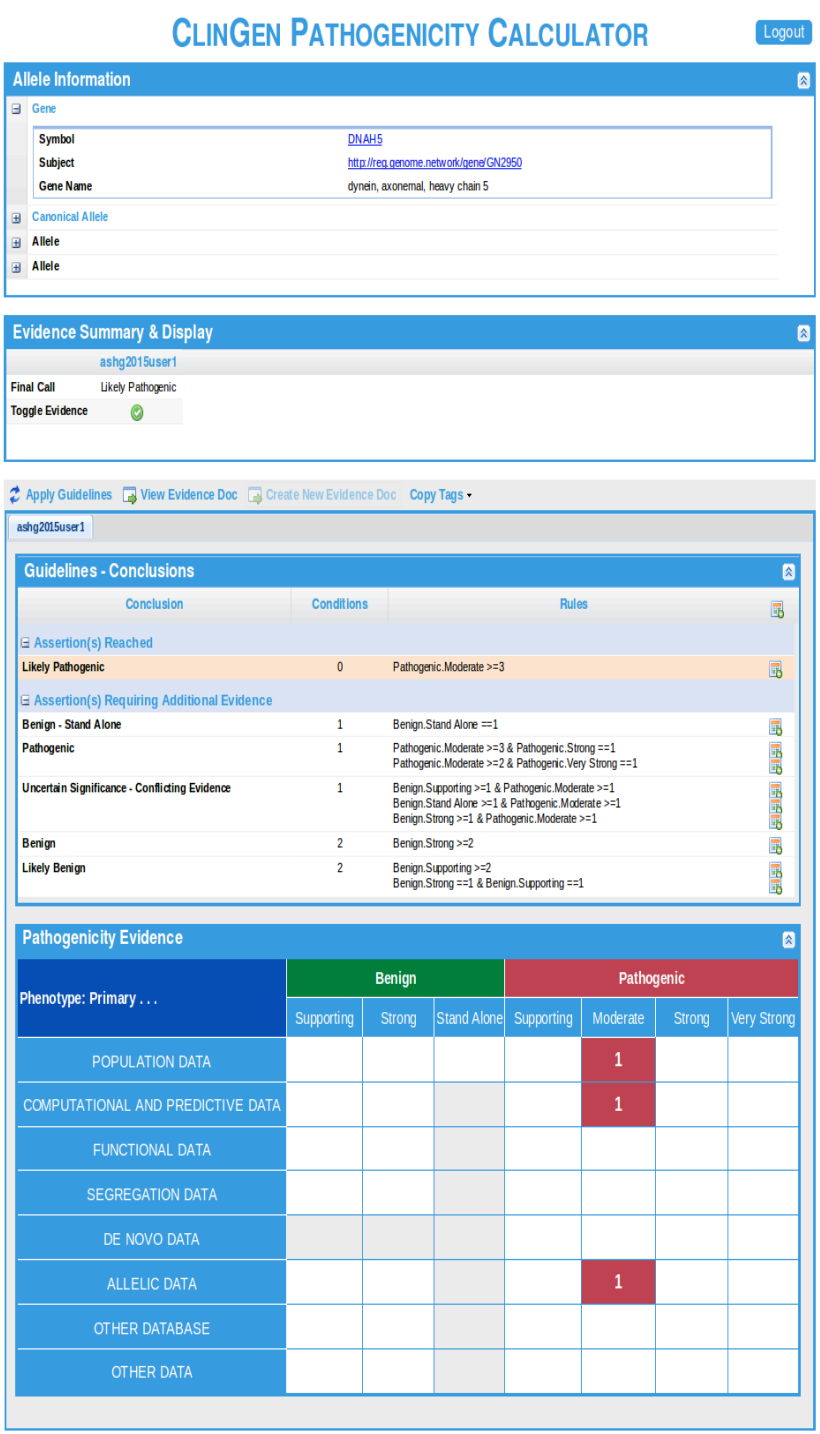
Turn the following evidence tags on:
PM2, PM3, PM4 for Primary ciliary dyskinesia

Use tag helper to locate the tags:
http://calculator.clinicalgenome.org/site/cg-grid-guide

Check the conclusion

Examine the rules applied to reach the conclusion.

Examine evidence that--if present--may lead to a different conclusion.



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Hailin Chen

Baylor College of Medicine

Sharon Plon

Rajarshi Ghosh

ClinGen
Clinical Genome Resource

NIH/NHGRI U01 HG007436

cser
Clinical Sequencing Exploratory Research

NIH/NHGRI U01 HG007307

DNAH5 (NM_001369.2): c.7468_7488del (p.Trp2490_Leu2496del)
Primary ciliary dyskinesia

Site	ACMG Rules	Lab Rules	PS1	PM2	PM3	PM4	PP3	PP4	PP5
Site 1	Uncertain Significance	Uncertain Significance		X		X	?	X	
Site 2	Uncertain Significance	Uncertain Significance		X		X			
Site 3	Uncertain Significance	Likely Pathogenic			X	X			
Site 4	Uncertain Significance	Likely Pathogenic		P	X	X			
Site 5	Likely Pathogenic	Uncertain Significance		X	P	X			X
Site 6	Likely Pathogenic	Likely Pathogenic		X		X	X	X	
Site 7	Likely Pathogenic	Likely Pathogenic		X	X	X			
Site 8	Likely Pathogenic	Likely Pathogenic	X		X	X		X	
Site 9	Likely Pathogenic	Likely Pathogenic		X		X		X	X

PS1 – Same amino acid change as an established pathogenic variant

No (must be different nucleotide and "established pathogenic")

PM2 - Absent in pop. databases

No (can't assume long indels in pop dbs) →But yes with ClinSeq data review

PM4 - Protein length changing variant

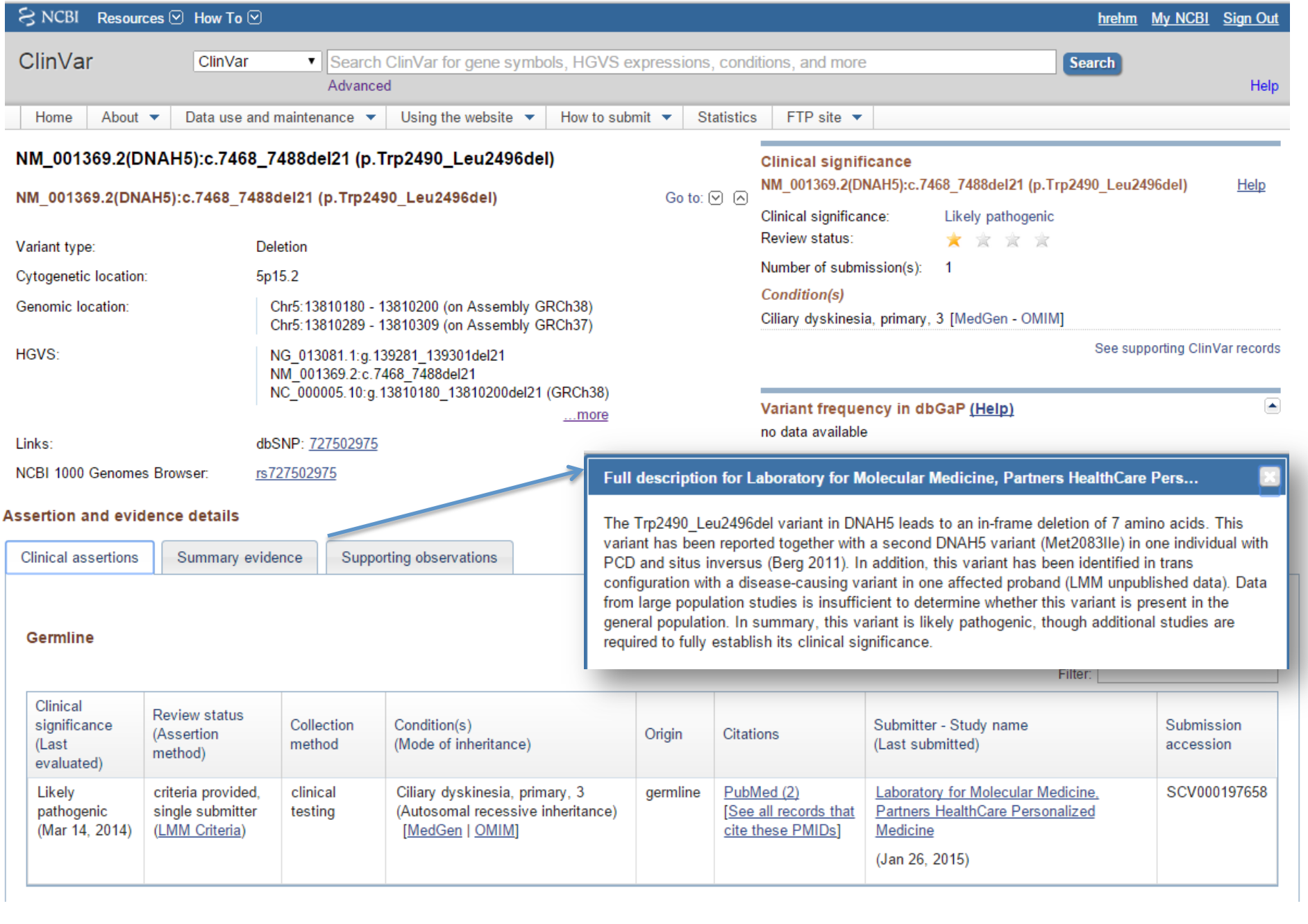
Yes

PP3 - Multiple lines of computational evidence

No (must be "all")

PP5 - Reputable source = pathogenic

No – only use if evidence not available and likely novel


NCBI Resources How To
hrehm My NCBI Sign Out
ClinVar
ClinVar
Search ClinVar for gene symbols, HGVS expressions, conditions, and more
Search
Advanced
Help
Home About Data use and maintenance Using the website How to submit Statistics FTP site
NM_001369.2(DNAH5):c.7468_7488del21 (p.Trp2490_Leu2496del)
NM_001369.2(DNAH5):c.7468_7488del21 (p.Trp2490_Leu2496del)
Go to:
Variant type: Deletion
Cytogenetic location: 5p15.2
Genomic location: Chr5:13810180 - 13810200 (on Assembly GRCh38)
Chr5:13810289 - 13810309 (on Assembly GRCh37)
HGVS: NG_013081.1:g.139281_139301del21
NM_001369.2:c.7468_7488del21
NC_000005.10:g.13810180_13810200del21 (GRCh38)
...more
Links: dbSNP: 727502975
NCBI 1000 Genomes Browser: rs727502975
Clinical significance
NM_001369.2(DNAH5):c.7468_7488del21 (p.Trp2490_Leu2496del)
Help
Clinical significance: Likely pathogenic
Review status:
Number of submission(s): 1
Condition(s)
Ciliary dyskinesia, primary, 3 [MedGen - OMIM]
See supporting ClinVar records
Variant frequency in dbGaP (Help)
no data available
Assertion and evidence details
Clinical assertions
Summary evidence
Supporting observations
Full description for Laboratory for Molecular Medicine, Partners HealthCare Pers...
The Trp2490_Leu2496del variant in DNAH5 leads to an in-frame deletion of 7 amino acids. This variant has been reported together with a second DNAH5 variant (Met2083Ile) in one individual with PCD and situs inversus (Berg 2011). In addition, this variant has been identified in trans configuration with a disease-causing variant in one affected proband (LMM unpublished data). Data from large population studies is insufficient to determine whether this variant is present in the general population. In summary, this variant is likely pathogenic, though additional studies are required to fully establish its clinical significance.
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
Likely pathogenic (Mar 14, 2014)
criteria provided, single submitter (LMM Criteria)
clinical testing
Ciliary dyskinesia, primary, 3 (Autosomal recessive inheritance) [MedGen | OMIM]
germline
PubMed (2) [See all records that cite these PMIDs]
Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine (Jan 26, 2015)
SCV000197658

DNAH5 (NM_001369.2): c.7468_7488del (p.Trp2490_Leu2496del)
Primary ciliary dyskinesia

Site	ACMG Rules	Lab Rules	PS1	PM2	PM3	PM4	PP3	PP4	PP5
Site 1	Uncertain Significance	Uncertain Significance		X		X	?	X	
Site 2	Uncertain Significance	Uncertain Significance		X		X			
Site 3	Uncertain Significance	Likely Pathogenic			X	X			
Site 4	Uncertain Significance	Likely Pathogenic		P	X	X			
Site 5	Likely Pathogenic	Uncertain Significance		X	P	X			X
Site 6	Likely Pathogenic	Likely Pathogenic		X		X	X	X	
Site 7	Likely Pathogenic	Likely Pathogenic		X	X	X			
Site 8	Likely Pathogenic	Likely Pathogenic	X		X	X		X	
Site 9	Likely Pathogenic	Likely Pathogenic		X		X		X	X

Yes

PM3 - Detected in *trans* with a pathogenic variant

This evidence was not found by all sites in LMM's ClinVar entry and in Berg publication.

Through consensus we agreed on 2 instances of *trans* observation.

Decided guidance needed on how to count strength of more than 1 *trans* observation.

PP4 - Patient's phenotype or FH highly specific for gene

Disagreement on whether this rule applied.

LMM Case: "Suspected diagnosis of primary ciliary dyskinesia"; situs inversus totalis, nasal biopsy suggestive of PCD;

Berg case: neonatal respiratory distress, bronchiectasis, situs inversus, sinusitis, frequent otitis media, and outer dynein arm defect observed on EM

DNAH5 (NM_001369.2): c.7468_7488del (p.Trp2490_Leu2496del
Primary ciliary dyskinesia

Final

Site	ACMG Rules	Lab Rules	Post Discussion	PS1	PM2	PM3	PM4	PP3	PP4	PP5
Site 1	Uncertain Significance	Uncertain Significance	Uncertain Significance		X		X	?	X	
Site 2	Uncertain Significance	Uncertain Significance	Uncertain Significance		X		X			
Site 3	Uncertain Significance	Likely Pathogenic	Likely Pathogenic			X	X			
Site 4	Uncertain Significance	Likely Pathogenic	Likely Pathogenic		P	X	X			
Site 5	Likely Pathogenic	Uncertain Significance	Uncertain Significance		X	P	X			X
Site 6	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic		X		X	X	X	
Site 7	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic		X	X	X			
Site 8	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic	X		X	X		X	
Site 9	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic		X		X		X	X
Consensus	**Likely Pathogenic**		**Not reached**		**X**	**X**	**X**		**Mixed**	
					*	*	*			

PM2 – Absent from pop db

PM3 - Detected in *trans* with a pathogenic variant

PM4 - Protein length changing variant

ACMG rules:
3 moderate = Likely Pathogenic

- 6 sites agree and 3 sites remain skeptical and stay with VUS classification
- Note: ACMG rules allow for professional judgement to overrule calculated class

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1

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2

Make sure you are in Slide Show mode

Still not working? Get help at pollev.com/app/help

or

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Take Home Points

- Variant classification often requires professional judgment and therefore complete consensus may not occur – this is OK!
- But, all evidence must be accessible and rules should be applied correctly
- And, it is useful for patients and physicians to have access to all opinions on a variant so……

Submit your classified variants to ClinVar!

- When we find differences, we can all work to resolve them and improvbe patient care.
- For those we don't understand, we can provide a source for others to decipher their effects

Acknowledgements

CSER Bake-Off Project (Laura Amendola et al.)

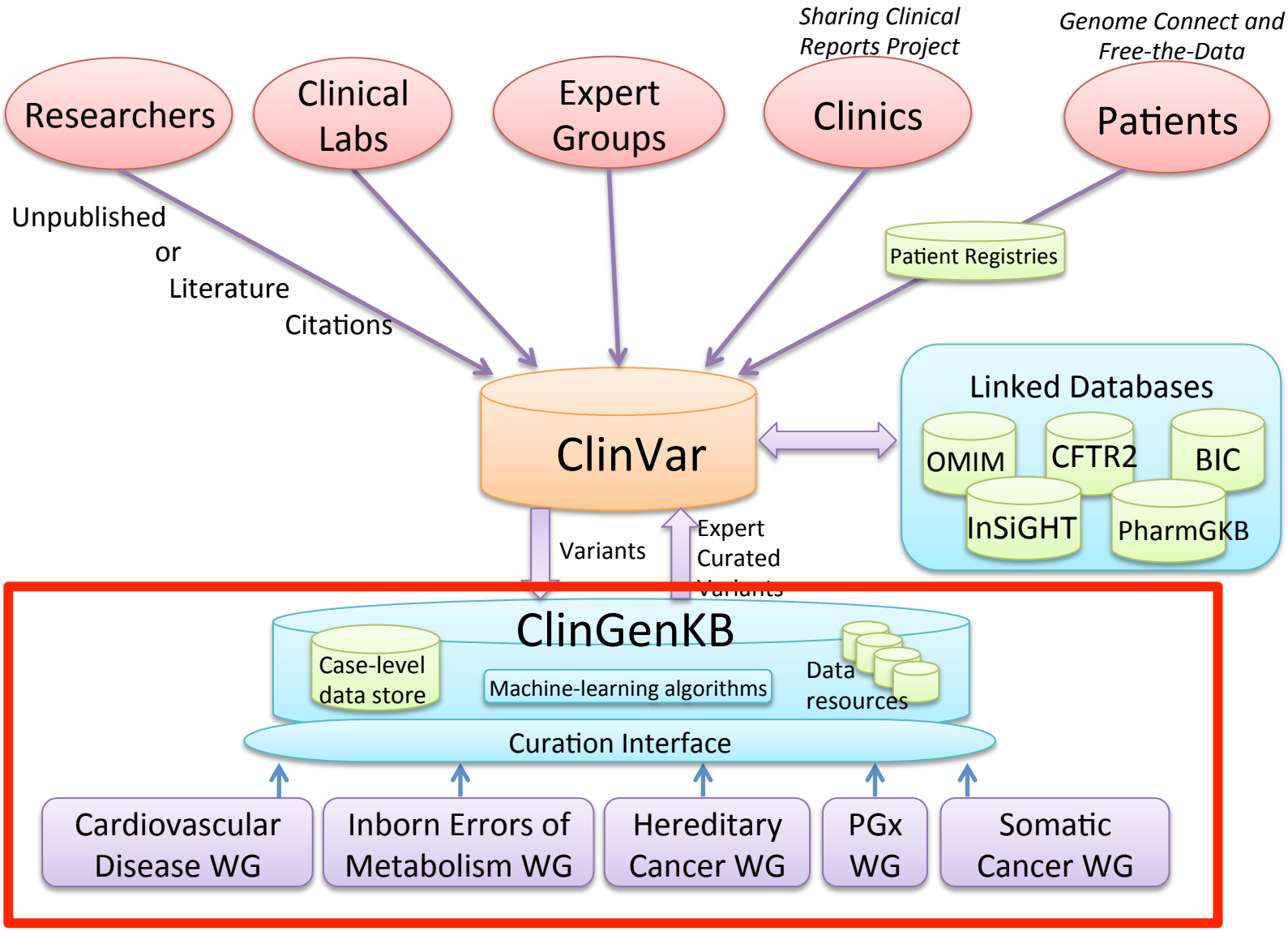
ACMG Interpreting Sequence Variants Working Group

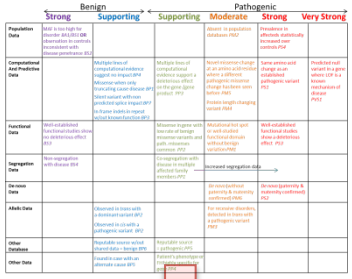
The ClinVar Staff at NCBI

ClinGen

Appendix

Supporting a Curation Environment for both Crowd-Sourcing and Expert Consensus





ACMG Rules

Interlab Seq Var Discrepancy Resolution Task Team

Cardiomyopathy Expert Panel

Noonan Spectrum Expert Panel

PTEN Expert Panel

Metabolic Disease Expert Panel

Developmental Delay Pilot Project

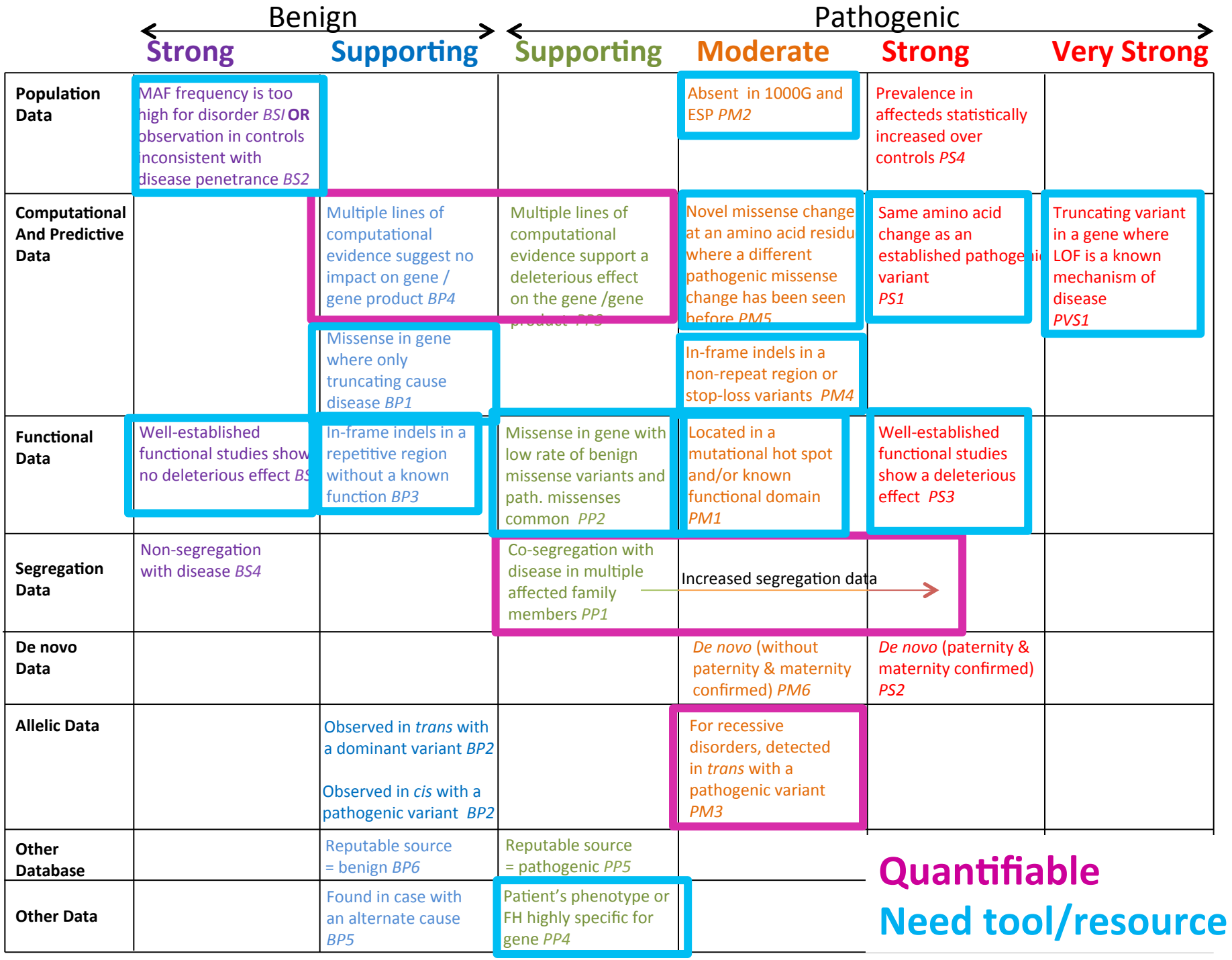
Congenital Muscular Dystrophy Pilot Project

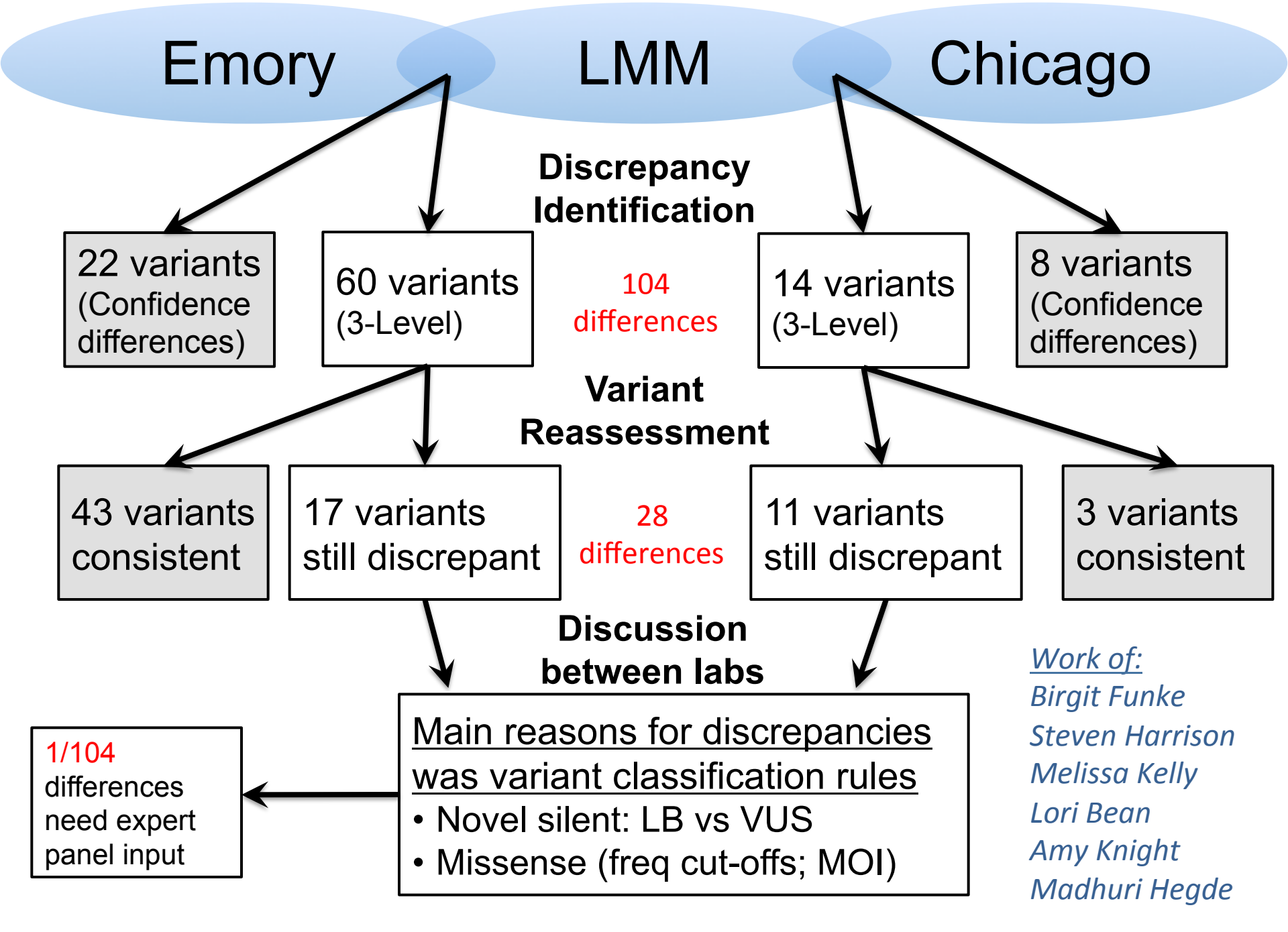
Each expert panel provides gene and disease-specific recommendations for ACMG rule specification (frequency thresholds, acceptable functional assays, define genes/regions for certain rule usage, etc)

ClinGen Sequence Variant Interpretation Work Group

(Co-Chairs Les Beisecker and Marc Greenblat)

1. Review and harmonize requested specifications from expert panels
2. Develop more quantitative approaches to enhance objective use of ACMG guidelines (segregation, multiple occurrences of the same rules; computational approaches; Baysesian models; multiple likelihood ratios)


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 product PP3
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 pathoge 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 BS
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

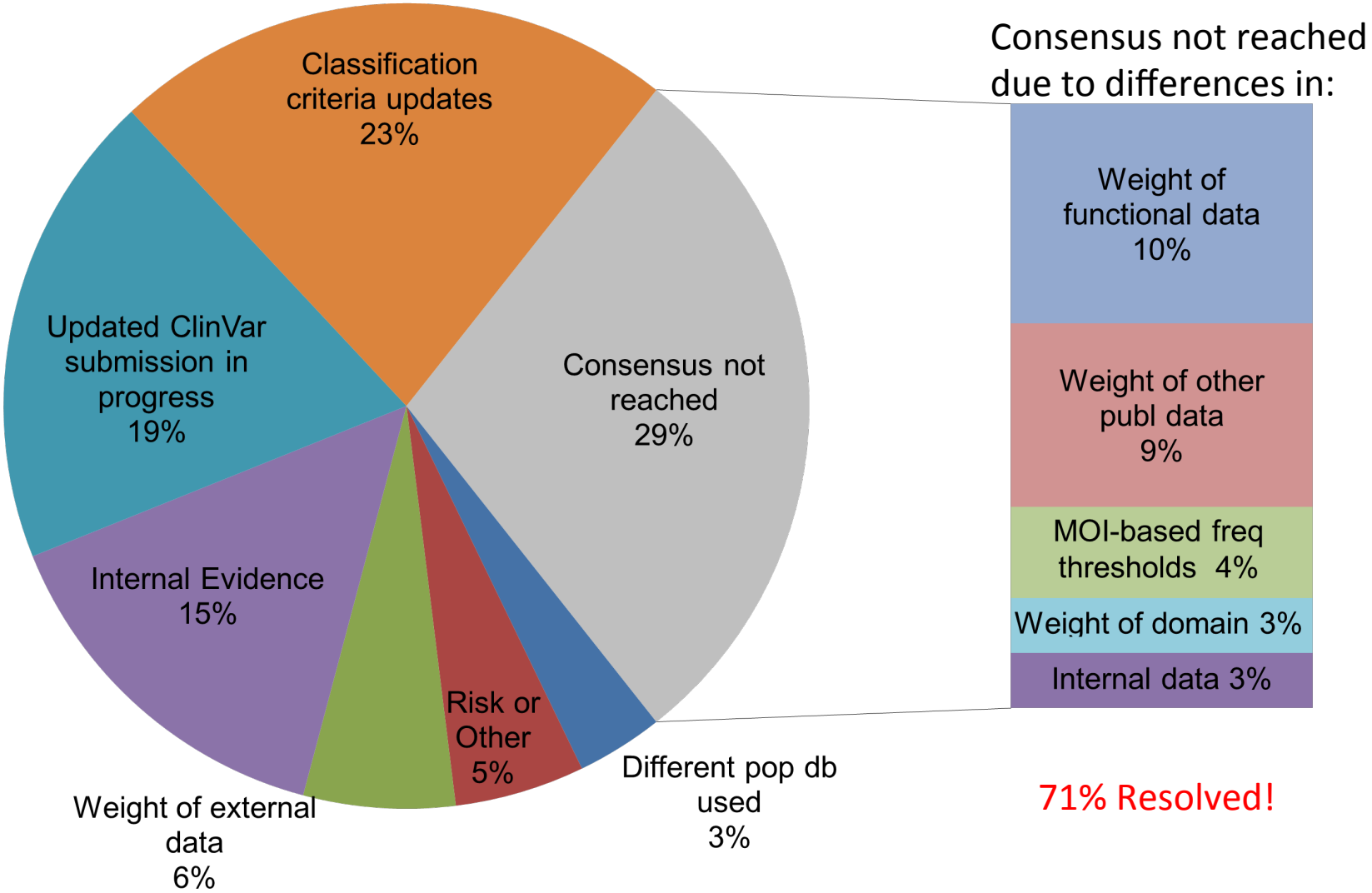

Emory
LMM
Chicago
Discrepancy Identification
22 variants (Confidence differences)
60 variants (3-Level)
104 differences
14 variants (3-Level)
8 variants (Confidence differences)
Variant Reassessment
43 variants consistent
17 variants still discrepant
28 differences
11 variants still discrepant
3 variants consistent
Discussion between labs
Main reasons for discrepancies was variant classification rules
• Novel silent: LB vs VUS
• Missense (freq cut-offs; MOI)
1/104 differences need expert panel input
Work of:
Birgit Funke
Steven Harrison
Melissa Kelly
Lori Bean
Amy Knight
Madhuri Hegde

Comparison of ClinVar Submitted Variants Across Four Labs: Ambry, GeneDx, Partners LMM, Univ. Chicago

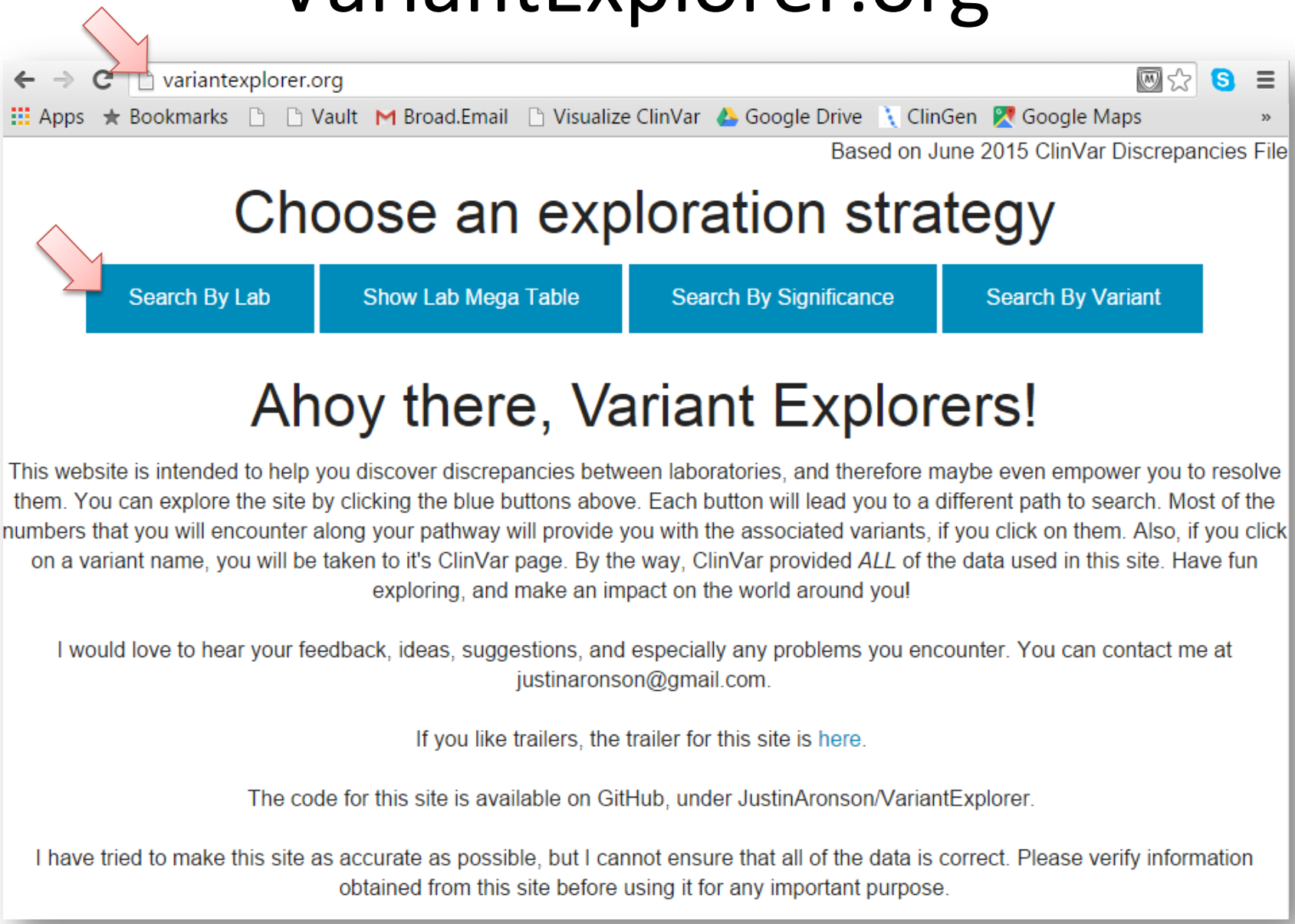
Submitted by	# shared variants	# Agreed (%)	# VUS to LB/ B differences	# actionable differences
Site A/Site D	2246	1993 (89%)	207 (9%)	46 (2%)
Site D/Site B	1793	1534 (86%)	61 (3%)	197 (11%)
Site C/Site B	463	422 (91%)	36 (8%)	5 (1%)
Site A/Site C	43	41 (95%)	2 (5%)	0
Site A/Site B	63	60 (95%)	2 (35)	1 (2%)
Site D/Site C	914	835 (91%)	79 (95)	0
All 4 Labs	**4878**	**4253 (87%)**	375 (8%)	250 (5%)

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

Basis for Interpretation Differences and Resolution Outcome of 115 Discordant Variants



VariantExplorer.org



James R. Lupski Lab,Baylor College of Medicine

Juha Muilu Group Institute for Molecular Medicine Finland (FIMM)

King Faisal Specialist Hospital and Research Center

LabCorp

Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine

Martin Pollak Beth Israel Deaconess Medical Center, Dept. of Nephrology

Medical Genetics Laboratories,Baylor College of Medicine

Molecular Genetics Diagnostic Laboratory,Children's

Significance Break Downs

All Other Labs:

Significance Name	Significance Variant Count	Pathogenic	Likely pathogenic	Uncertain significance	Likely benign	Benign
Pathogenic	Coming Soon	0	49	4	0	0
Likely pathogenic	Coming Soon	144	0	10	0	1
Uncertain significance	Coming Soon	180	128	0	43	31
Likely benign	Coming Soon	19	7	197	0	284
Benign	Coming Soon	16	8	78	305	0

NM_000256.3(MYBPC3):c.3628-41_3628-17del

Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine	GeneDx,GeneDx
Likely pathogenic (Cardiomyopathy, familial hypertrophic, 4)	Benign (Cardiomyopathy)
Dec 4 2013 12:00:00:000AM	Jan 7 2014 12:00:00:000AM
SCV000203933	SCV000208326
The 3628-41_3628-17del variant in MYBPC3 has been reported in multiple individuals with HCM or unspecified cardiomyopathy (Waldmuller 2003, Tanjore 2008, Dhandapany 2009). These studies showed that this variant alters splicing and leads to skipping of exon 33 (Waldmuller 2003, Dhandapany 2009). This is consistent with a disease-causing role based on the high prevalence of splice variants in HCM patients. In addition, cell culture studies showed an effect on sarcomere architecture (Dhandapany 2009). This variant is particularly prevalent in populations of South Asian ancestry, where it was found in 2-8% of individuals	Although c.3628-41_3628-17del has been reported as a possible risk factor for common adult-onset heart conditions including cardiomyopathy (Waldmuller S et al., 2003; Dhandapany P et al., 2009; Srivastava A et al., 2011), this variant is listed in the SNP database (rs36212066) and is present in 1-3% of Southeast Asian control alleles

Choose an exploration strategy

Search By Lab | Show Lab Mega Table | Search By Significance | Search By Variant

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	3	2	5
Ambry Genetics	2	7	9
Biesecker Laboratory - ClinSeq Project, NHGRI	0	2	2
Blueprint Genetics	38	14	52
CSER_CC_NCGL	19	5	24
Cardiovascular Biomedical Research Unit Royal Brompton & Harefield NHS Foundation Trust	30	3	33
Counsyl	0	18	18
Department of Ophthalmology and Visual Sciences Kyoto University	0	5	5
Emory Genetics Laboratory	101	38	139
Evolutionary and Medical Genetics Laboratory, Centre for Cellular and Molecular Biology	2	0	2
GeneDx,GeneDx	236	305	541
GeneReviews	7	4	11
Genetic Services Laboratory, University of Chicago	35	205	240
Genomic Research Center, Shahid Beheshti University of Medical Sciences	2	0	2
Greenwood Genetic Center Diagnostic Laboratories,Greenwood Genetic Center	4	0	4
InSiGHT	1	3	4