

ClinGen Pathogenicity Calculator:

Use case *GLA*

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

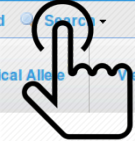
In the present use case, these four evidence tags will be used for this allele to calculate conclusion based on ACMG guidelines

Step 1: Identify allele: Click on search

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid

Canonical Allele	Views	Evidence	ACMG							Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	



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

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG								Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	Undetermined	
<div style="border: 1px solid #ccc; padding: 10px; width: fit-content; margin: 0 auto;"><p>Allele Search ✕</p><p>HGVS Term: <input type="text" value="Type HGVS term ..."/></p><p style="text-align: center;"><input type="button" value="Search"/> <input type="button" value="Cancel"/> <input type="button" value="Reset"/></p></div>											

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

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

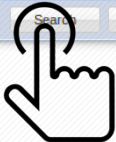
CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG							Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	

Allele Search [X]

HGVS Term:




Step 1: Identify allele: View search results

CLINGEN PATHOGENICITY CALCULATOR

Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG								Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	Undetermined	
CA021883		0	0	0	0	0	0	0	0	0	0



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

CLINGEN PATHOGENICITY CALCULATOR

Logout


Clear Grid Search

Canonical Allele	Views	Evidence	ACMG							Non ACMG	
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence		Undetermined
CA021883		0	0	0	0	0	0	0	0	0	0
Allele Name	Nucleotide Change	Simple Allele Type									
NM_000169.2:c.639+919G>A	SO:1000002*substitution	transcript									
NC_000023.11:g.101399747C>T	SO:1000002*substitution	genomic									
NM_000169.2:c.640-801G>A	SO:1000002*substitution	transcript									

Step 2: Launch the calculator

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG							Non ACMG	
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence		Undetermined
CA021883		0	0	0	0	0	0	0	0	0	0

Click on the calculator icon

Learn more about gene/
allele

CLINGEN PATHOGENICITY CALCULATOR

Logout


Allele Information

Gene

Canonical Allele

Allele


Allele



Evidence Summary & Display

ashg2015user1

Final Call

Toggle Evidence 

 Apply Guidelines  View Evidence Doc  Create New Evidence Doc [Copy Tags](#) ▾

No tabs are currently active.
Click the **Toggle Evidence** cell on [Evidence Summary & Display](#) Panel to activate a tab.

CLINGEN PATHOGENICITY CALCULATOR

Logout

Allele Information

Gene

Symbol	GLA
Subject	http://reg.genome.network/gene/GN4296
Gene Name	galactosidase, alpha

Canonical Allele


Allele

HGVS	NM_000169.2:c.639+919G>A
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Evidence Summary & Display

ashg2015user1

Final Call

Toggle Evidence 

Apply Guidelines View Evidence Doc Create New Evidence Doc Copy Tags

No tabs are currently active.
Click the **Toggle Evidence** cell on [Evidence Summary & Display](#) Panel to activate a tab.

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

CLINGEN PATHOGENICITY CALCULATOR

Logout

Allele Information

Gene	
Symbol	GLA
Subject	http://reg.genome.network/gene/GN4296
Gene Name	galactosidase, alpha
Canonical Allele	
Allele	
HGVS	NM_000169.2:c.639+919G>A

Evidence Summary & Display

ashg2015user1

Final Call


Toggle Evidence

[Apply Guidelines](#) [View Evidence Doc](#) [Create New Evidence Doc](#) [Copy Tags](#)

ashg2015user1

No Evidence

No Evidence for the tab.
Activate the **Create New Evidence Doc** button to make new evidence document.
Creating new evidence document will activate the "Guidelines - Conclusions" table.



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

Provide information about condition and mode of inheritance

CLINGEN PATHOGENICITY CALCULATOR Logout

Allele Information

Gene

Symbol	GLA
Subject	http://reg.genome.network/gene/GN4296
Gene Name	galactosidase, alpha

Canonical Allele

Allele

HGVS	NM_000169.2:c.639+919G>A
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Evidence Summary & Display

ashg2015user1

Final Call

Toggle Evidence

ashg2015user1

[Apply Guidelines](#) [View Evidence Doc](#)

No Evidence

Create New Evidence

Evidence will be provided for which phenotype?:
Fabry disease

What is the Mode of Inheritance?:
X-linked Recessive

Save Cancel Reset

No Evidence for the tab.
Activate the **Create New Evidence Doc** button to make new evidence document.
Creating new evidence document will activate the "Guidelines - Conclusions" table.

Step 3: Create evidence document and input evidence

Click OK to notification

CLINGEN PATHOGENICITY CALCULATOR Logout

Allele Information

Gene

Symbol: [GLA](#)

Subject: <http://ref.genome.network/gene/GN4296>

Gene Name: galactosidase, alpha

Canonical Allele

Allele

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

Evidence Summary & Display

ashg2015user1

Final Call: Undetermined

Toggle Evidence:

SUCCESS

New Evidence document CLI-CF5EM6-EV was created successfully. Please use the 'Pathogenicity Evidence' table to add tags.

OK

No Tags

No Tags for this evidence document.
Use the **Pathogenicity Table** below to add tags to the document.
Saving new tags to the documents will activate the "Guidelines - Conclusions" table.

Pathogenicity Evidence

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

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

Allele Information

Gene

Symbol: [GLA](#)

Subject: <http://reg.genome.network/gene/GN4296>

Gene Name: galactosidase, alpha

Canonical Allele

Allele

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

Evidence Summary & Display

ashg2015user1

Final Call: Undetermined

Toggle Evidence:

Apply Guidelines View Evidence Doc Create New Evidence Doc Copy Tags


ashg2015user1

No Tags

No Tags for the evidence document.
Use the **Pathogenicity Table** below to add tags to the document.
Saving new tags to the documents will activate the "Guidelines - Conclusions" table.

Pathogenicity Evidence

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



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

The screenshot displays the Clingen Pathogenicity Calculator interface. At the top, the "Allele Information" section shows details for the gene *GLA* (galactosidase, alpha) with the HGVS notation NM_000169.2:c.639>919G>A. Below this, the "Evidence Summary & Display" section shows the user "ashg2015user1" and the "Final Call" as "Undetermined".

The main interface includes a navigation bar with options: "Apply Guidelines", "View Evidence Doc", "Create New Evidence Doc", and "Copy Tags". The current view is titled "ashg2015user1" and "No Tags".

An "Evidence Tags for the cell: Pathogenic » Strong » Population Data" dialog box is open, showing a table with the following data:

Evidence Tag ID	Tag	Status	Link Summary	Summary
Tag PS4	PS4	On	No Links	Statistical difference in frequency case vs control

Buttons for "Update" and "Cancel" are visible at the bottom of the dialog. The background interface shows a sidebar with "Patho" and "Strong" categories, and a main table with the following rows:

COMPUTATIONAL AND PREDICTIVE DATA						
FUNCTIONAL DATA						
SEGREGATION DATA						
DE NOVO DATA						
ALLELIC DATA						
OTHER DATABASE						
OTHER DATA						

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

Allele Information

Gene

Symbol [GLA](#)

Subject <http://reg.genome.network/gene/GM4296>

Gene Name galactosidase, alpha

Canonical Allele

Allele

HGVS [NM_000169.2:c.639+919G>A](#)

Evidence Summary & Display

ashg2015user1

Final Call Pathogenic

Toggle Evidence

Apply Guidelines View Evidence Doc Create New Evidence Doc Copy Tags


ashg2015user1

Guidelines - Conclusions

Conclusion	Conditions	Rules
Assertion(s) Reached		
Pathogenic	0	Pathogenic.Strong >=2
Assertion(s) Requiring Additional Evidence		
Benign - Stand Alone	1	Benign.Stand Alone ==1
Uncertain Significance - Conflicting Evidence	1	Benign.Supporting >=1 & Pathogenic.Strong >=1 Benign.Stand Alone >=1 & Pathogenic.Strong >=1 Benign.Strong >=1 & Pathogenic.Strong >=1
Likely Pathogenic	*	Pathogenic.Moderate ==1 & Pathogenic.Strong ==1
Benign	2	Benign.Strong >=2
Likely Benign	2	Benign.Supporting >=2 Benign.Strong ==1 & Benign.Supporting ==1

Pathogenicity Evidence

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



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

Allele Information

- Gene
- Canonical Allele
- Allele
- Allele
- Allele

Evidence Summary & Display

ashg2015user1

Final Call: Pathogenic

Toggle Evidence:

Apply Guidelines | View Evidence Doc | Create New Evidence Doc | Copy Tags


ashg2015user1

Guidelines - Conclusions

Conclusion	Conditions	Rules
Assertion(s) Reached		
Pathogenic	0	Pathogenic.Strong >=2
Assertion(s) Requiring Additional Evidence		
Benign - Stand Alone	1	Benign.Stand Alone ==1
Uncertain Significance - Conflicting Evidence	1	Benign.Stand Alone >=1 & Pathogenic.Strong >=1 Benign.Strong >=1 & Pathogenic.Strong >=1 Benign.Supporting >=1 & Pathogenic.Strong >=1
Likely Pathogenic	*	Pathogenic.Moderate ==1 & Pathogenic.Strong ==1
Benign	2	Benign.Strong >=2
Likely Benign	2	Benign.Supporting >=2 Benign.Strong ==1 & Benign.Supporting ==1

Pathogenicity Evidence

Phenotype: Fabry disease	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							
OTHER DATA							



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

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid

Canonical Allele	Views	Evidence	ACMG							Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	


Click Search

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

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG						Non ACMG	
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence		Insuf. Evidence
Click HGVS										



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


CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

Canonical Allele	Views	Evidence	ACMG							Non ACMG
			Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence	

Allele Search

HGVS Term:

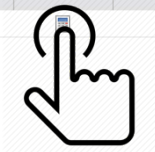


Enter HGVS and Click Search

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

CLINGEN PATHOGENICITY CALCULATOR Logout

Clear Grid Search

	Canonical Allele	Views	Evidence	ACMG							Non ACMG	
				Benign	Likely Benign	Benign Stand Alone	Pathogenic	Likely Pathogenic	Conflict. Evidence	Insuf. Evidence		Undetermined
CA021883			1	0	0	0	1	0	0	0	0	0

Step 5: Retrieve stored evidence and conclusion

Allele Information

- Gene
- Canonical Allele
- Allele
- Allele
- Allele

Evidence Summary & Display

ashg2015user1

Final Call: Pathogenic

Toggle Evidence:

Apply Guidelines View Evidence Doc Create New Evidence Doc Copy Tags

ashg2015user1

Guidelines - Conclusions

Conclusion	Conditions	Rules
Assertion(s) Reached		
Pathogenic	0	Pathogenic.Strong >=2
Assertion(s) Requiring Additional Evidence		
Benign - Stand Alone	1	Benign.Stand Alone ==1
Uncertain Significance - Conflicting Evidence	1	Benign.Supporting >=1 & Pathogenic.Supporting >=1 Benign.Supporting >=1 & Pathogenic.Strong >=1 Benign.Stand Alone >=1 & Pathogenic.Supporting >=1 Benign.Stand Alone >=1 & Pathogenic.Strong >=1 Benign.Strong >=1 & Pathogenic.Supporting >=1 Benign.Strong >=1 & Pathogenic.Strong >=1
Likely Pathogenic	*	Pathogenic.Strong ==1 & Pathogenic.Supporting >=2 Pathogenic.Moderate ==1 & Pathogenic.Strong ==1

Pathogenicity Evidence

Phenotype: Fabry disease	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							
OTHER DATA							

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