# 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)

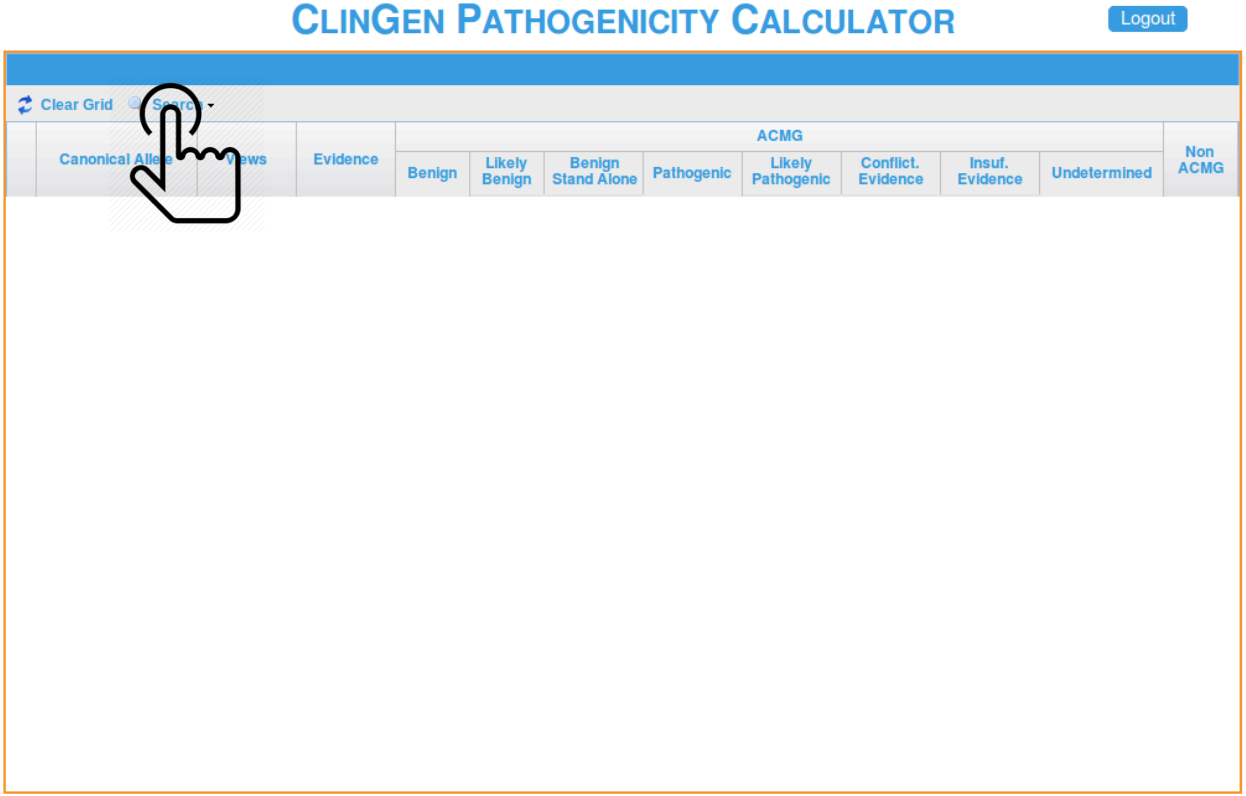
Three groups curated the variant with PP1-Moderate,PS3, PS4,PVS1, PM4,PP1,PP5, BP4,PP3 tags, leading to 3 different conclusions per ACMG Guidelines:

Pathogenic, Likely Pathogenic, Uncertain Significance

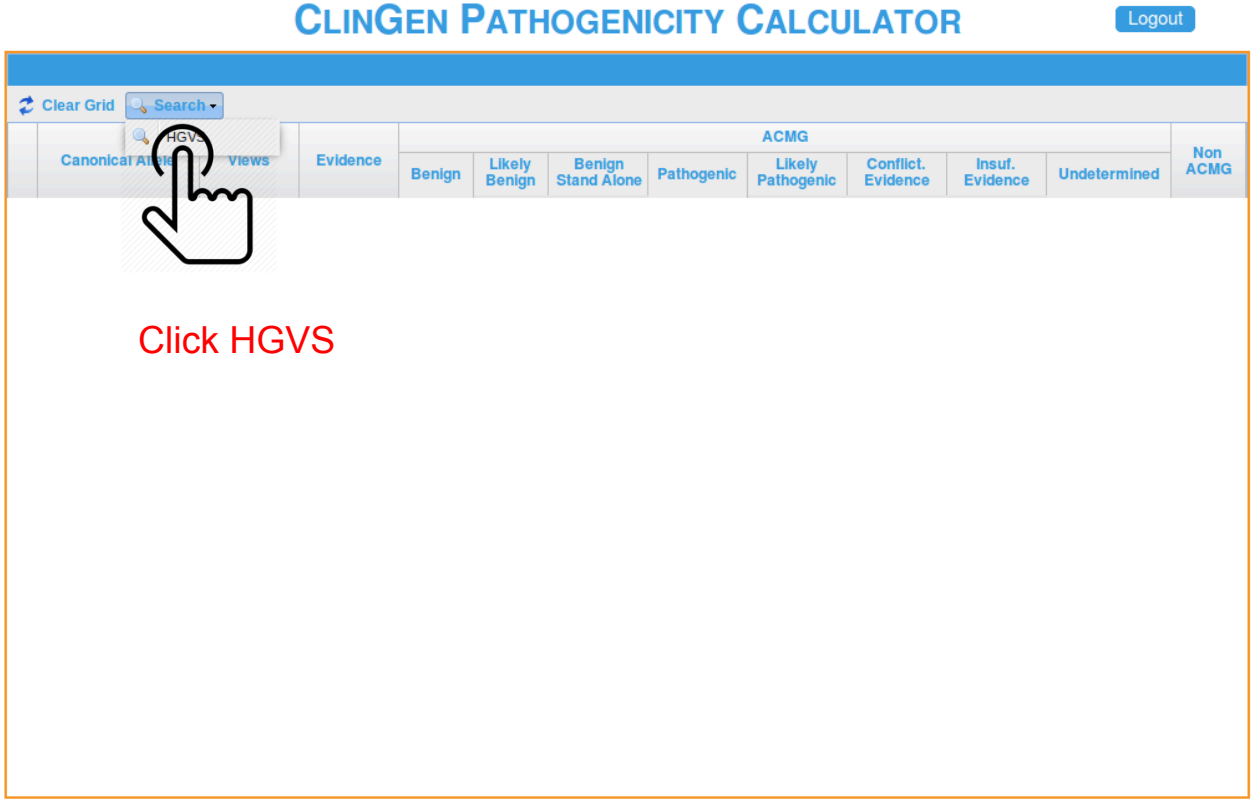
Consensus curation agreed on the following evidence tags for Fabry disease: PS4, PVS1-Strong, PS3, PP1

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

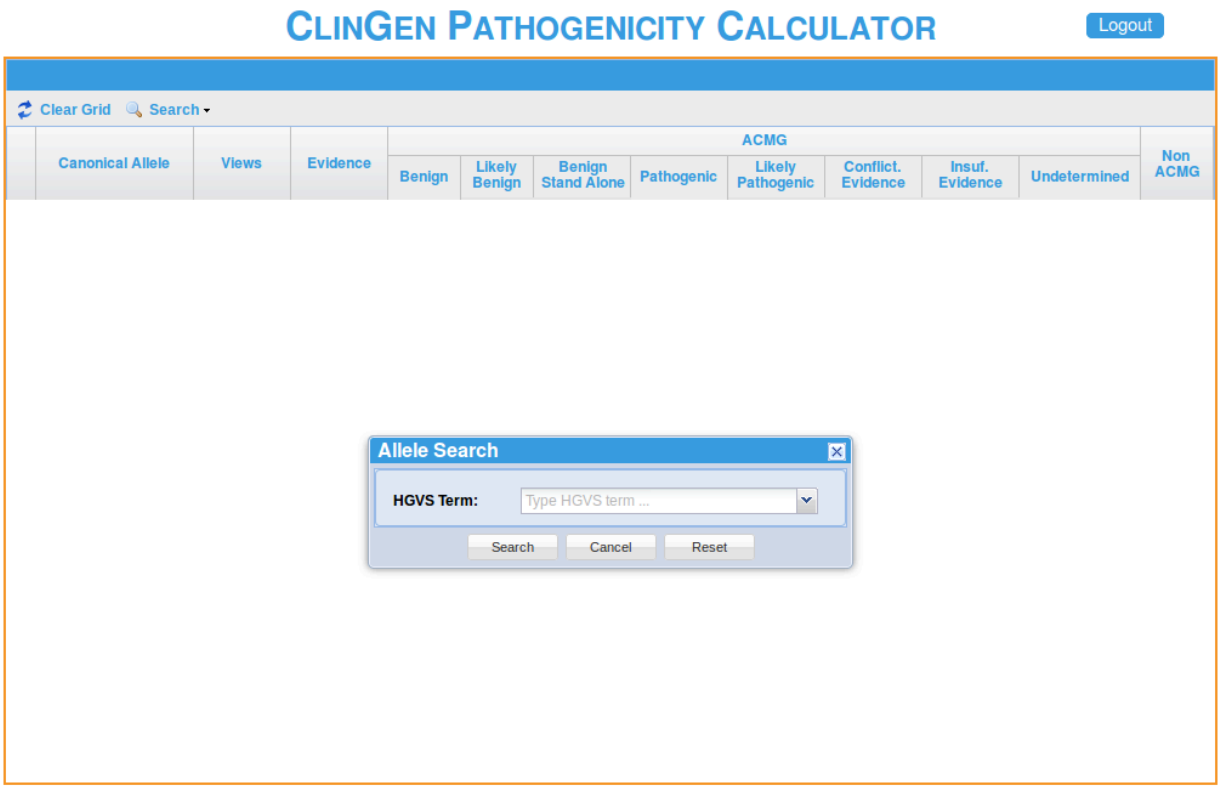
# Step 1: Identify allele: Click on search



# Step 1: Identify allele: Click on HGVS

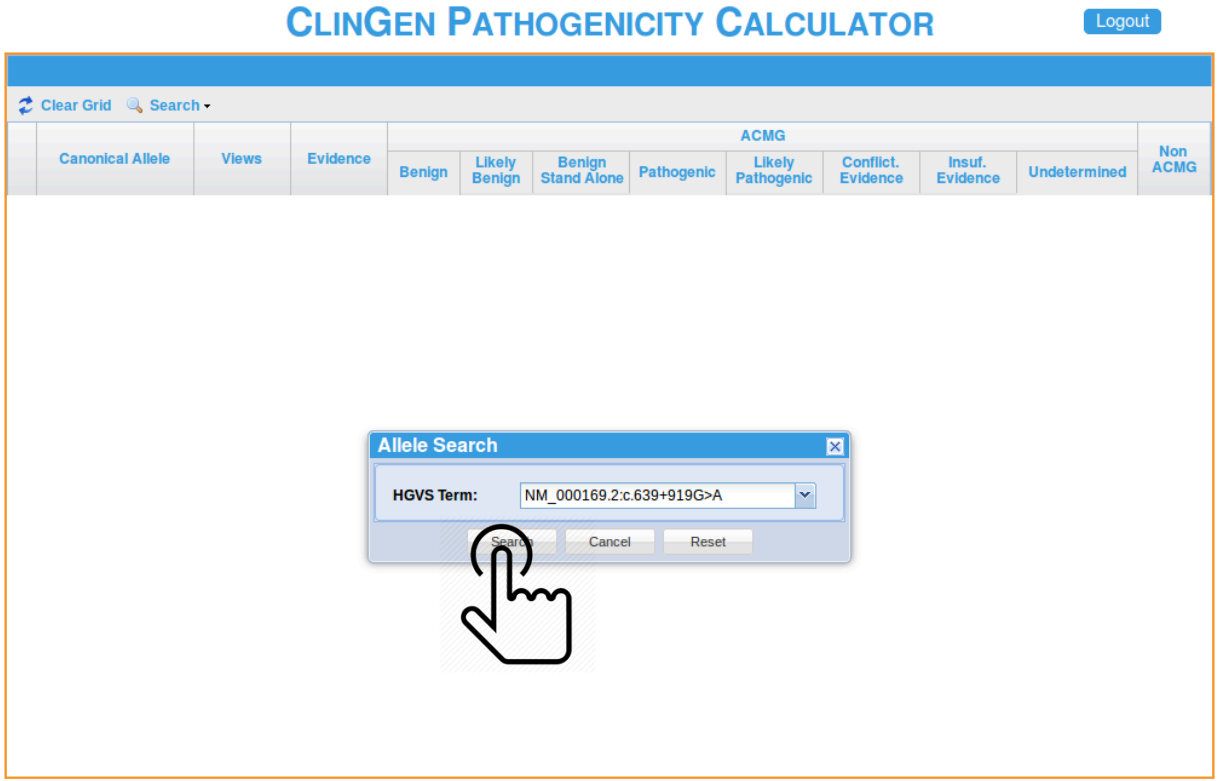


# Step 1: Identify allele: The allele Search panel pops up

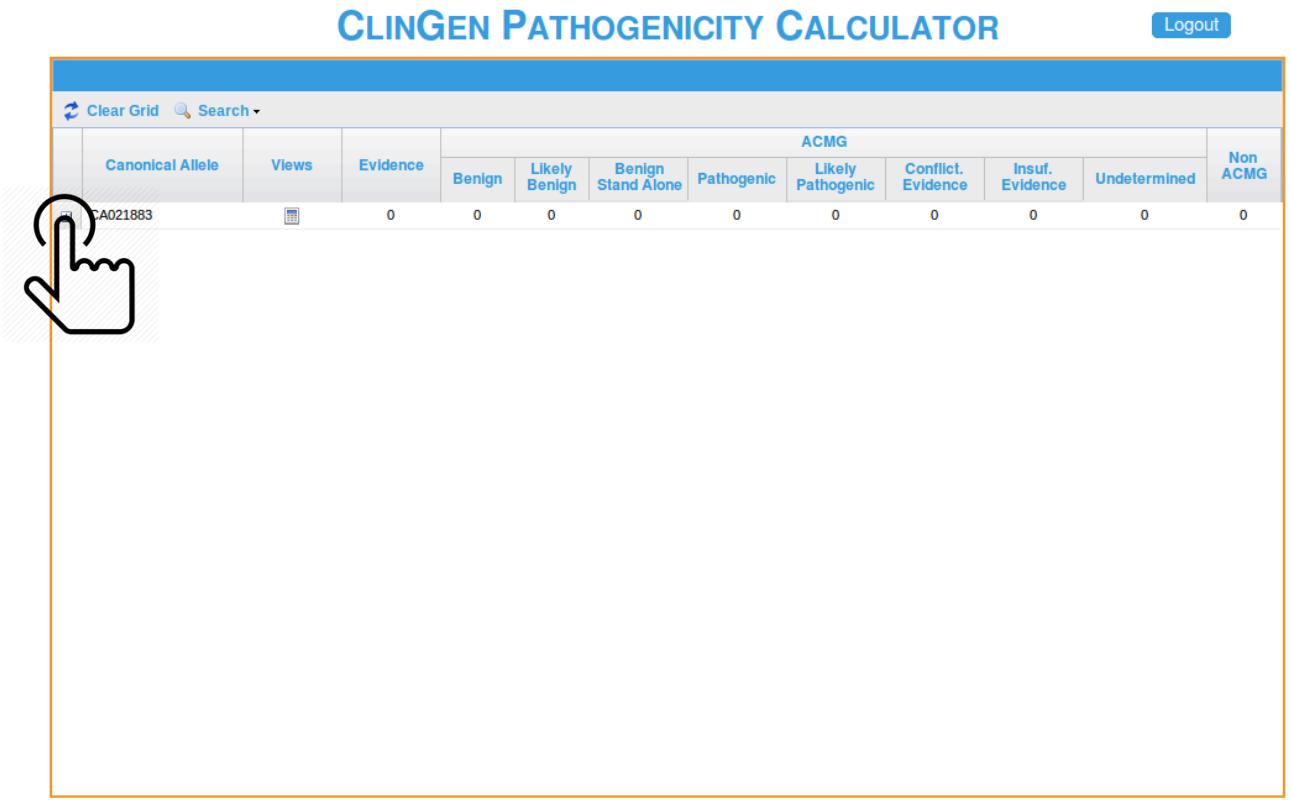


# Step 1: Identify allele: The allele search panel pops up.

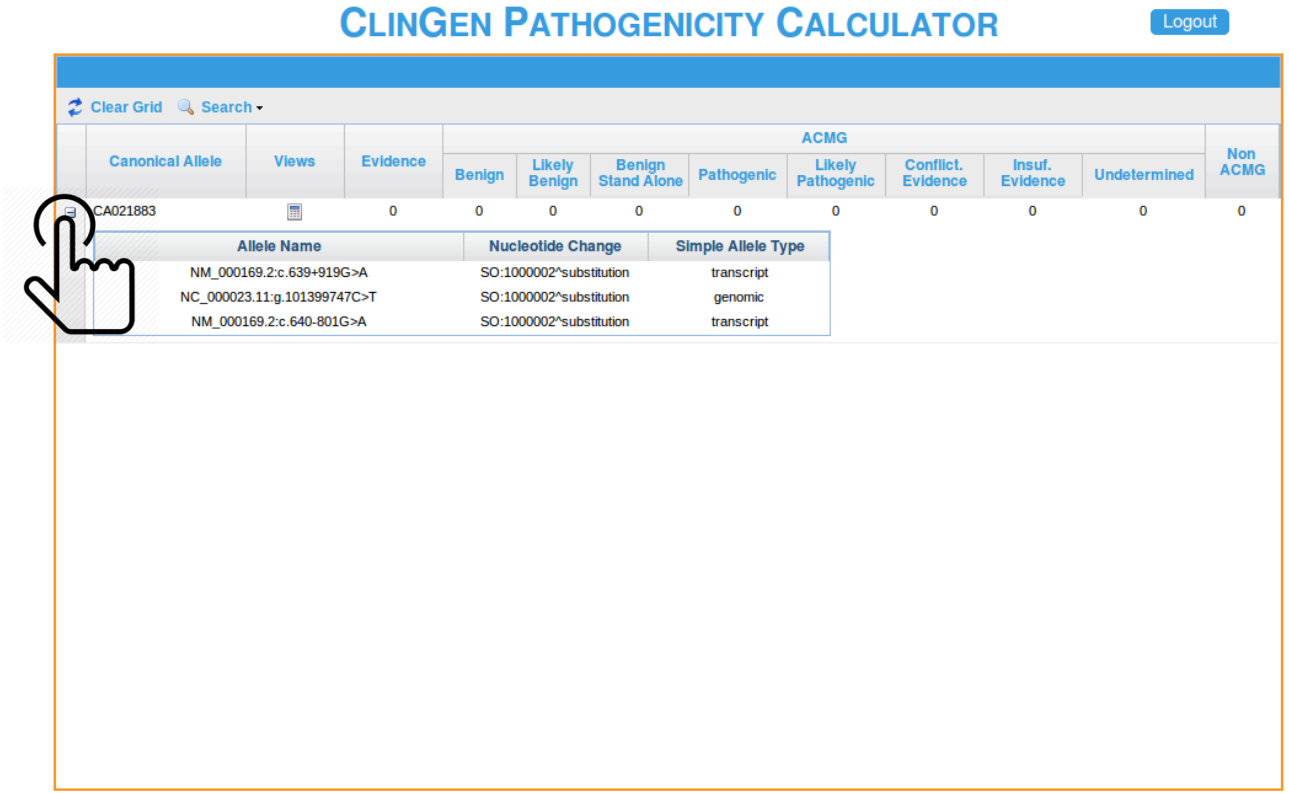
Search: **NM_000169.2:c.639+919G>A**



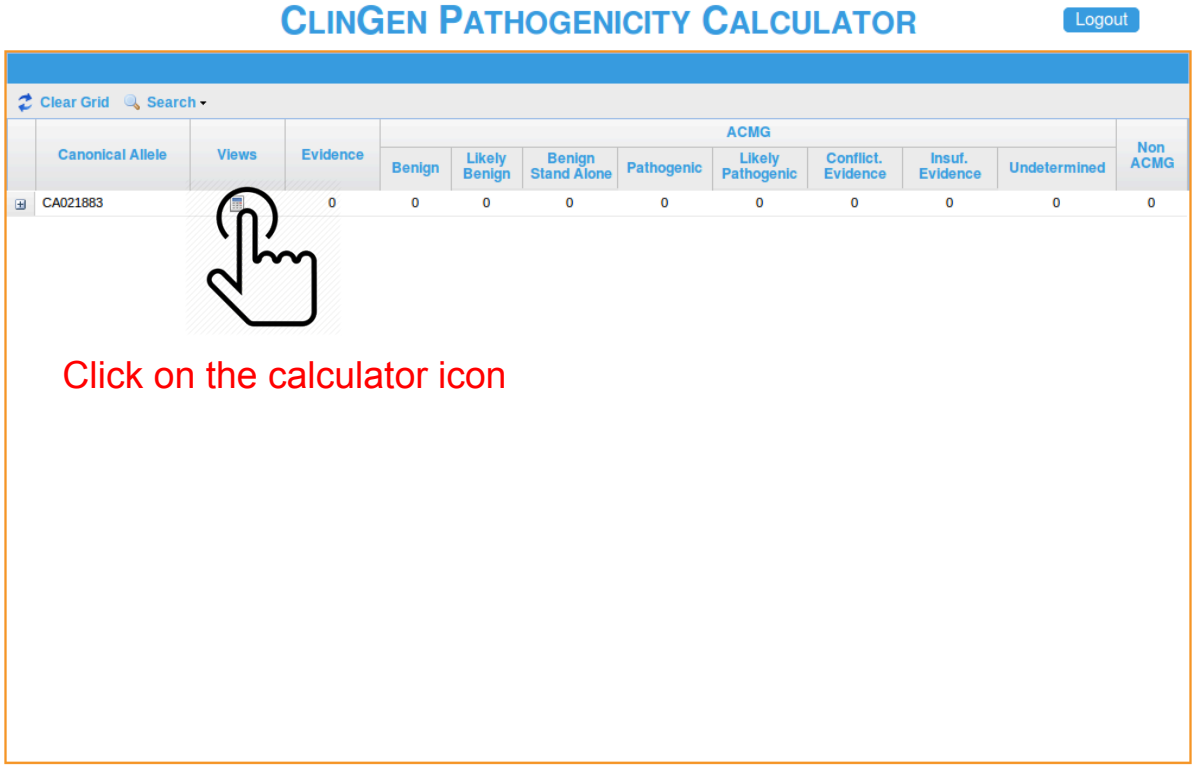
# Step 1: Identify allele: View search results



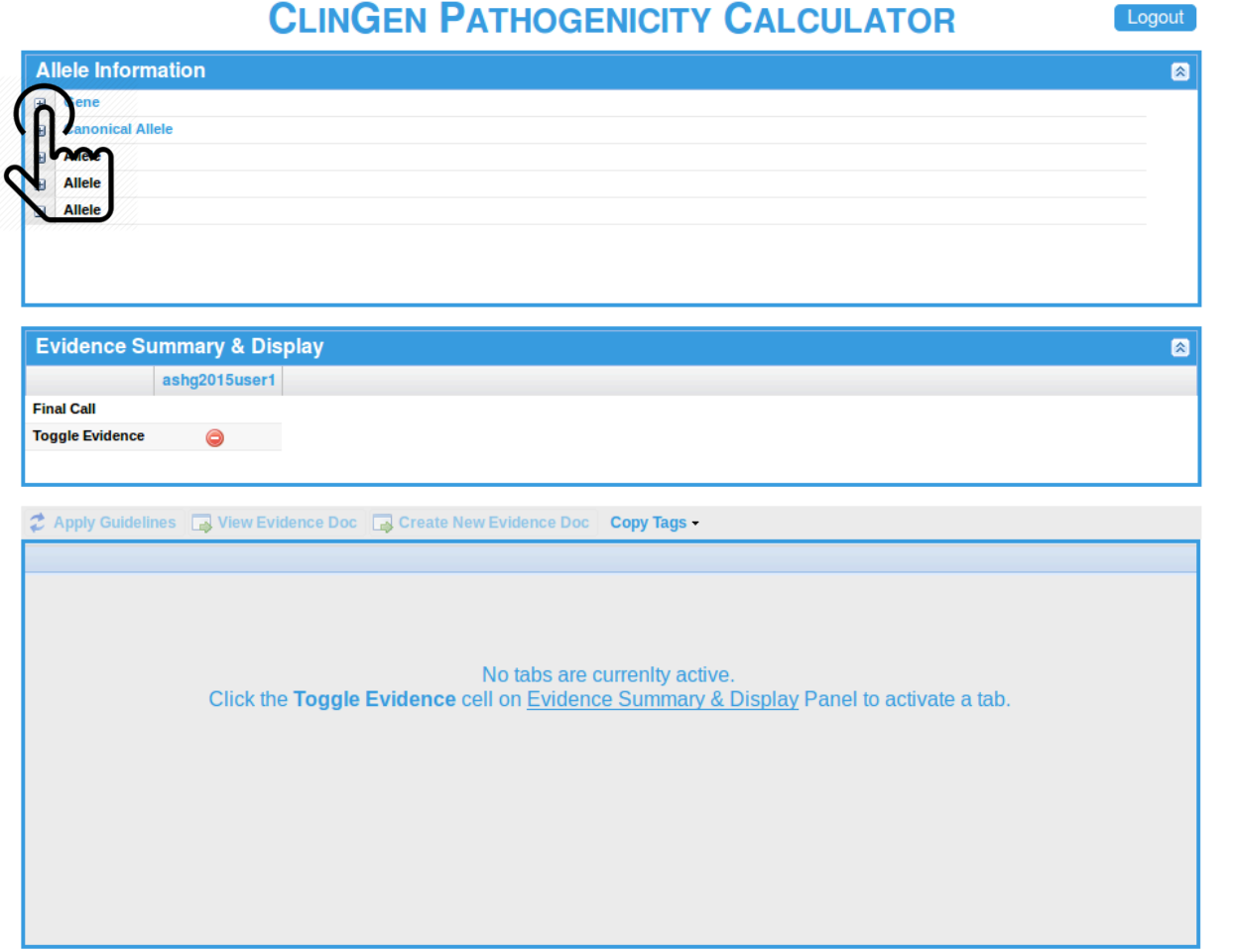
# Step 1: Identify allele: Inspect equivalent allele representations and confirm allele identity



# Step 2: Launch the calculator



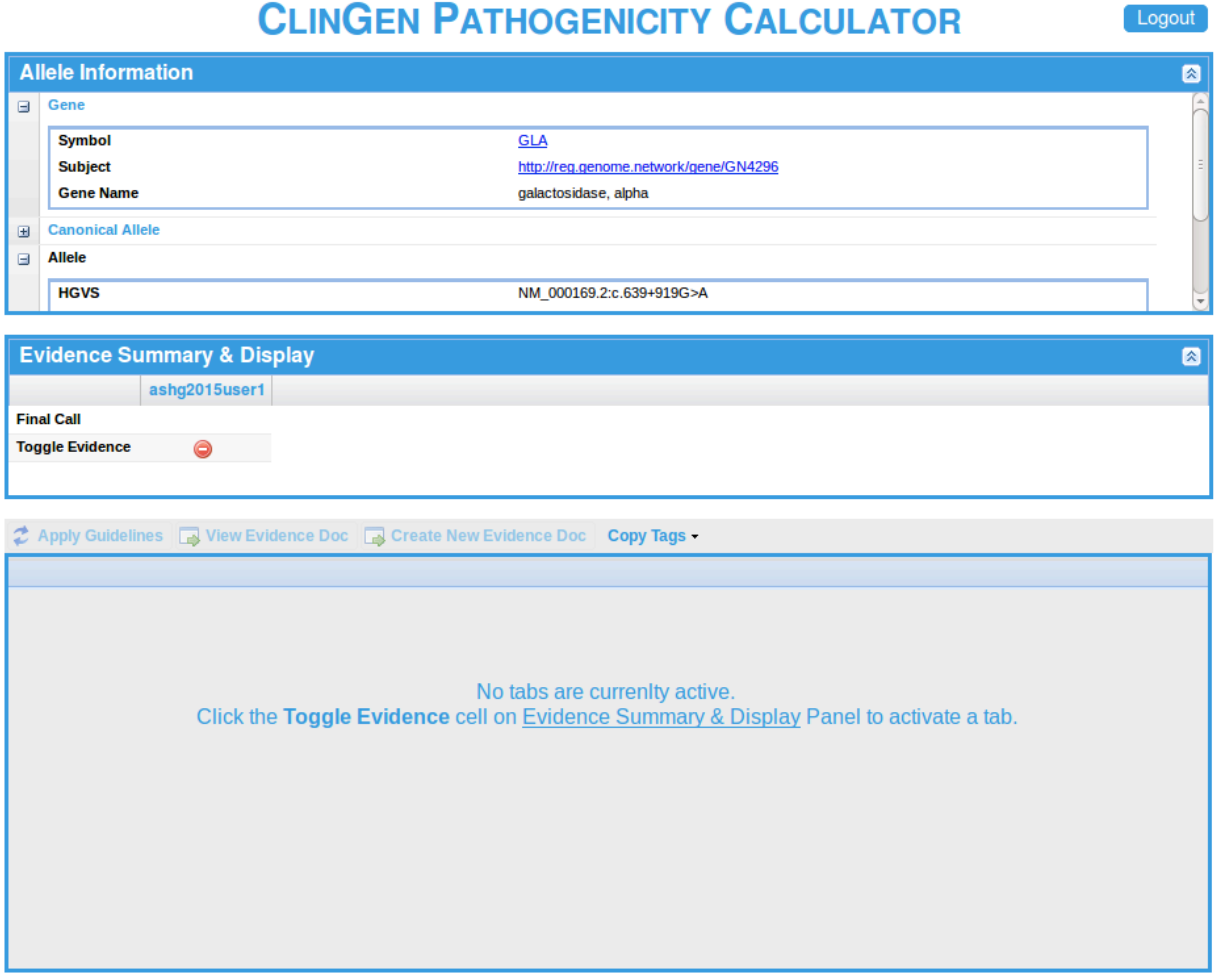
Learn more about gene/ allele



Step 2: Launch the calculator: Open the calculator tab

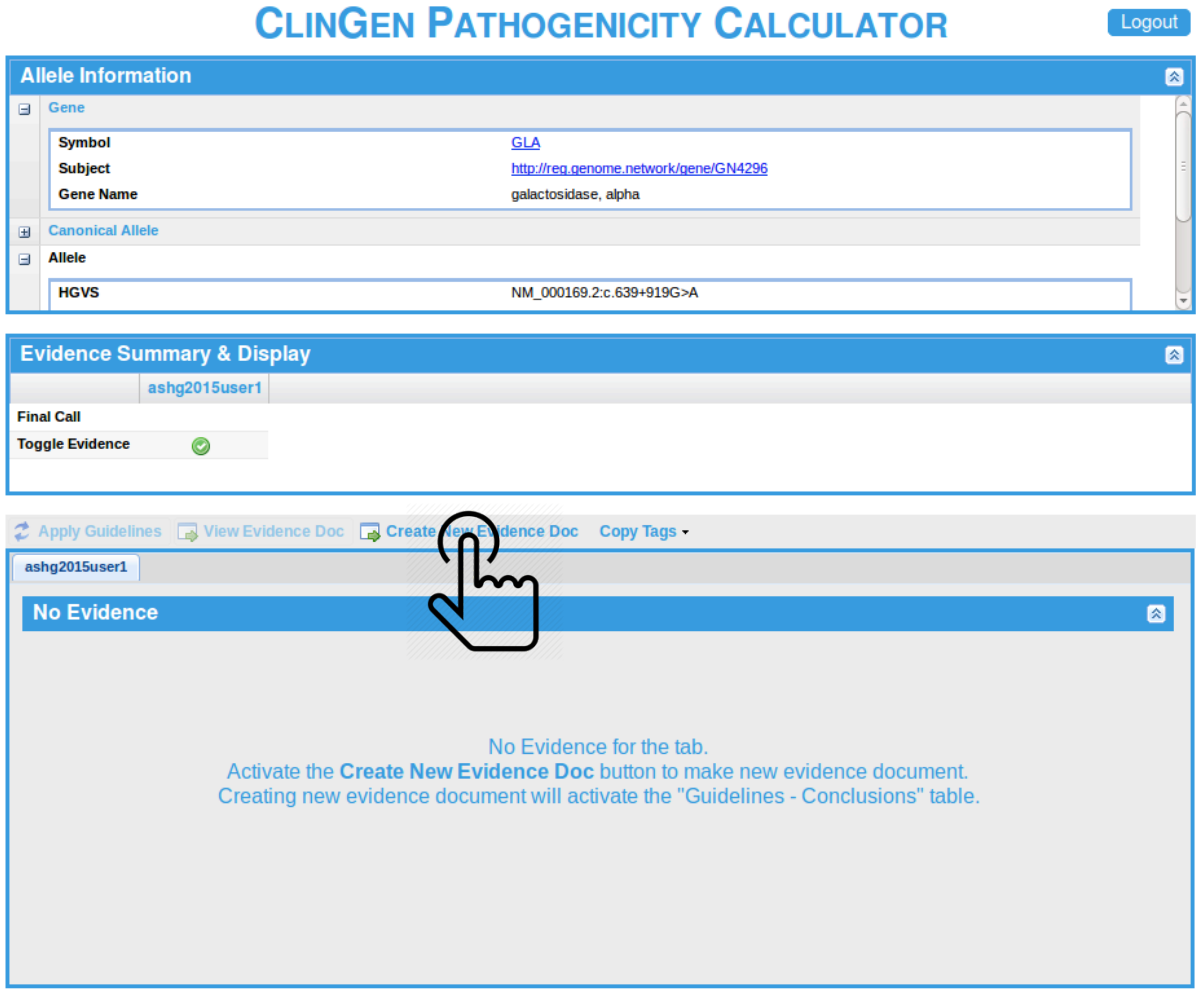
Because the evidence document is empty, the tab is not displayed

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



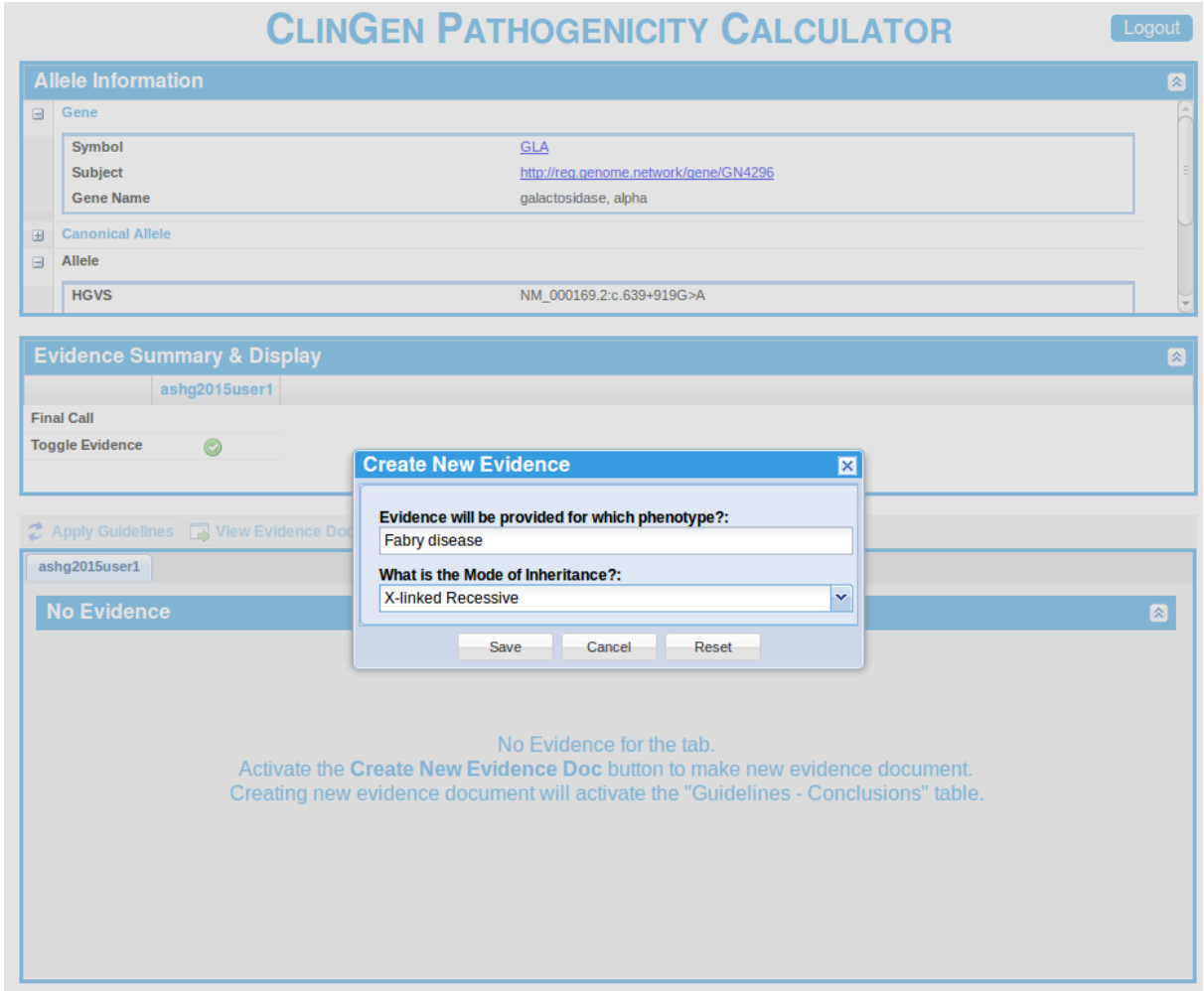
# Step 3: Create evidence document and input evidence

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



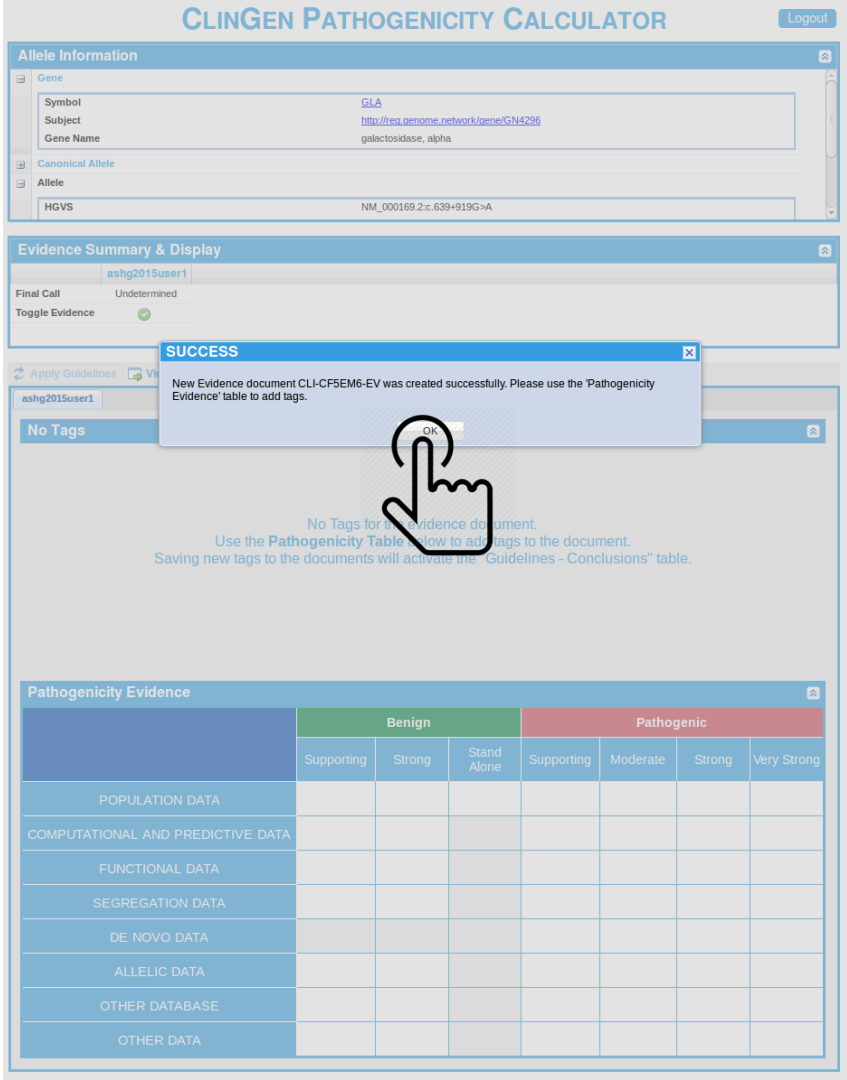
## Step 3: Create evidence document and input evidence: Provide basic information

Provide information about condition and mode of inheritance

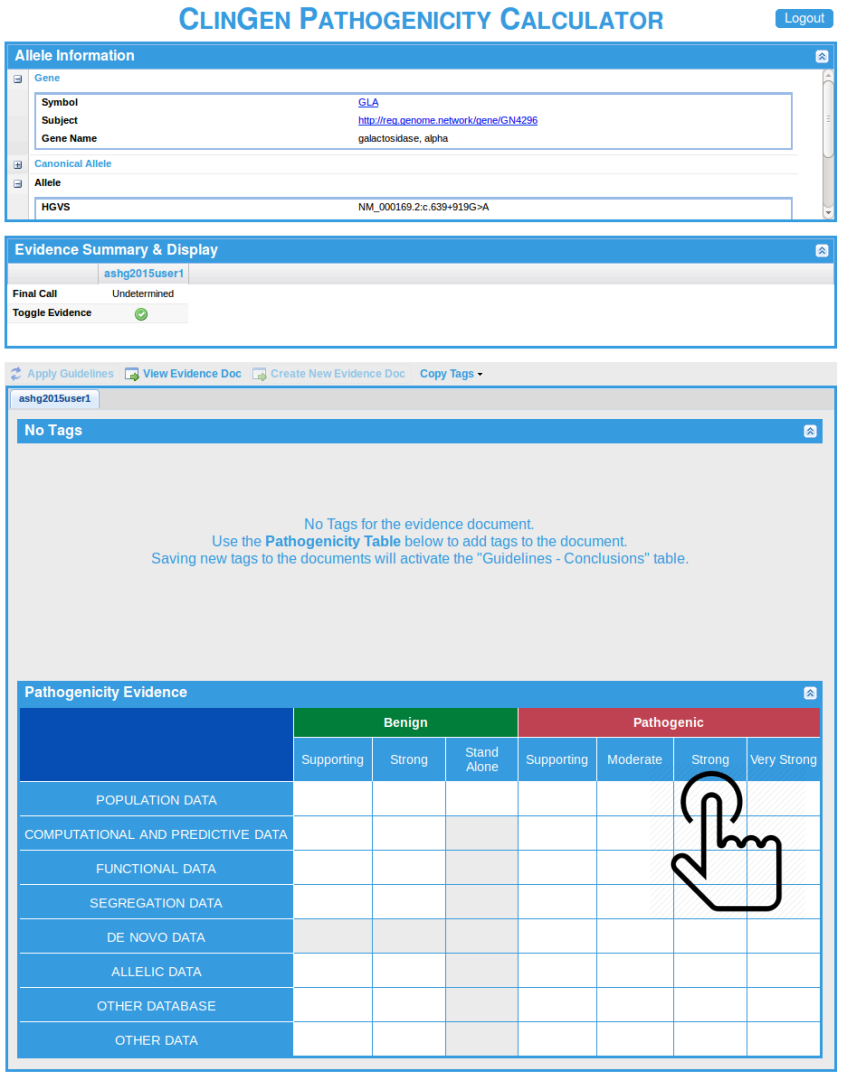


Step 3: Create evidence document and input evidence

**Click OK to notification**

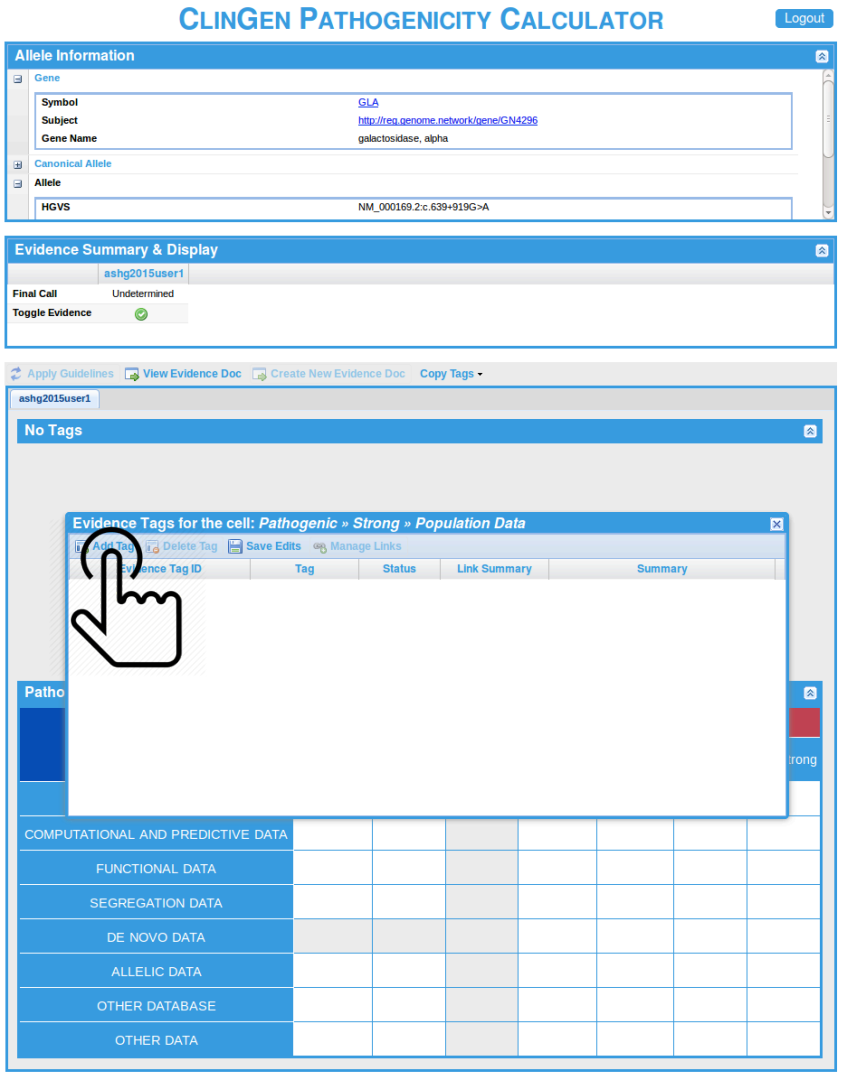


# Step 3: Create evidence document and input evidence: Turn PS4 tag on



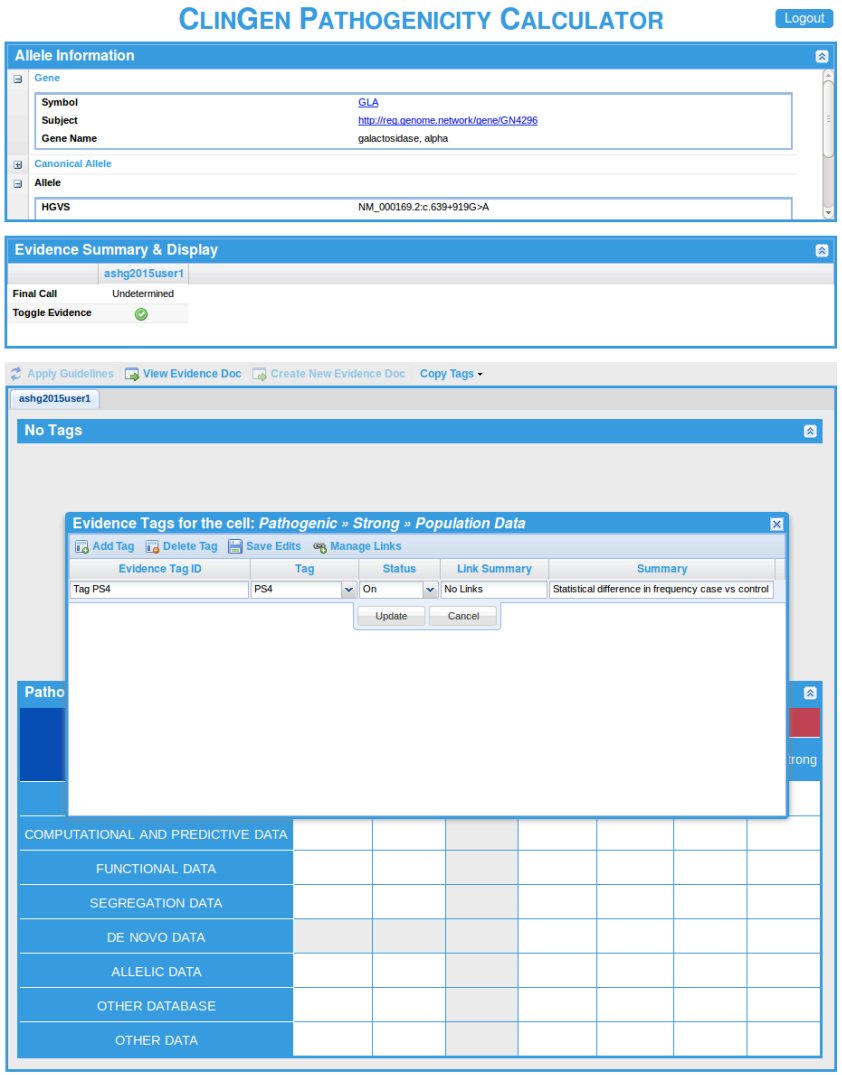
# Step 3: Create evidence document and input evidence: Turn PS4 tag on

Click Add Tag

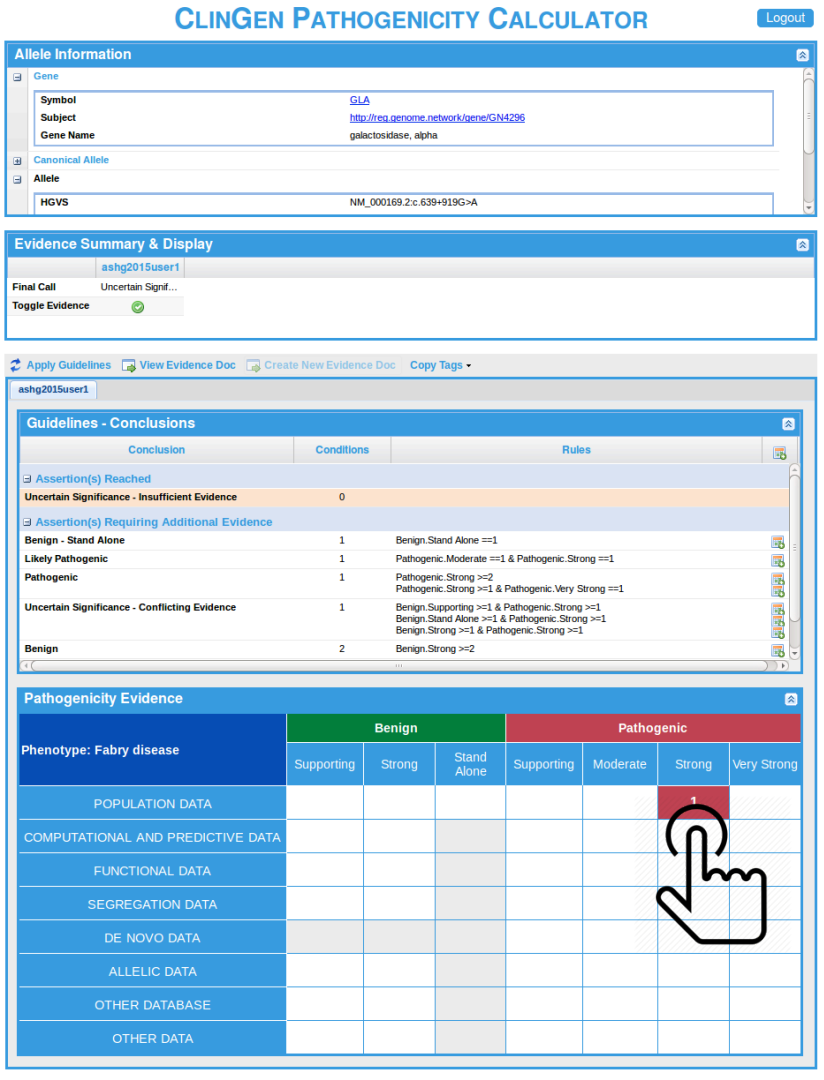


# Step 3: Create evidence document and input evidence: Turn PS4 tag on

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

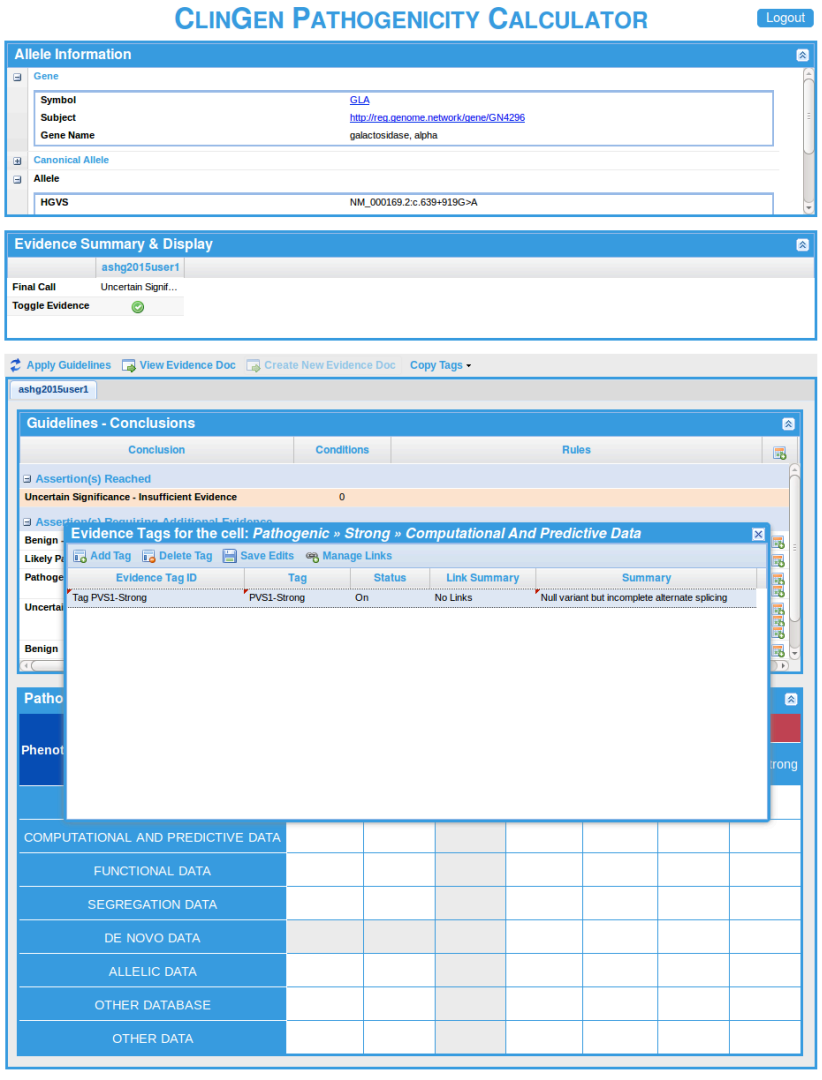


# Step 3: Create evidence document and input evidence: Turn PVS1-Strong tag ON

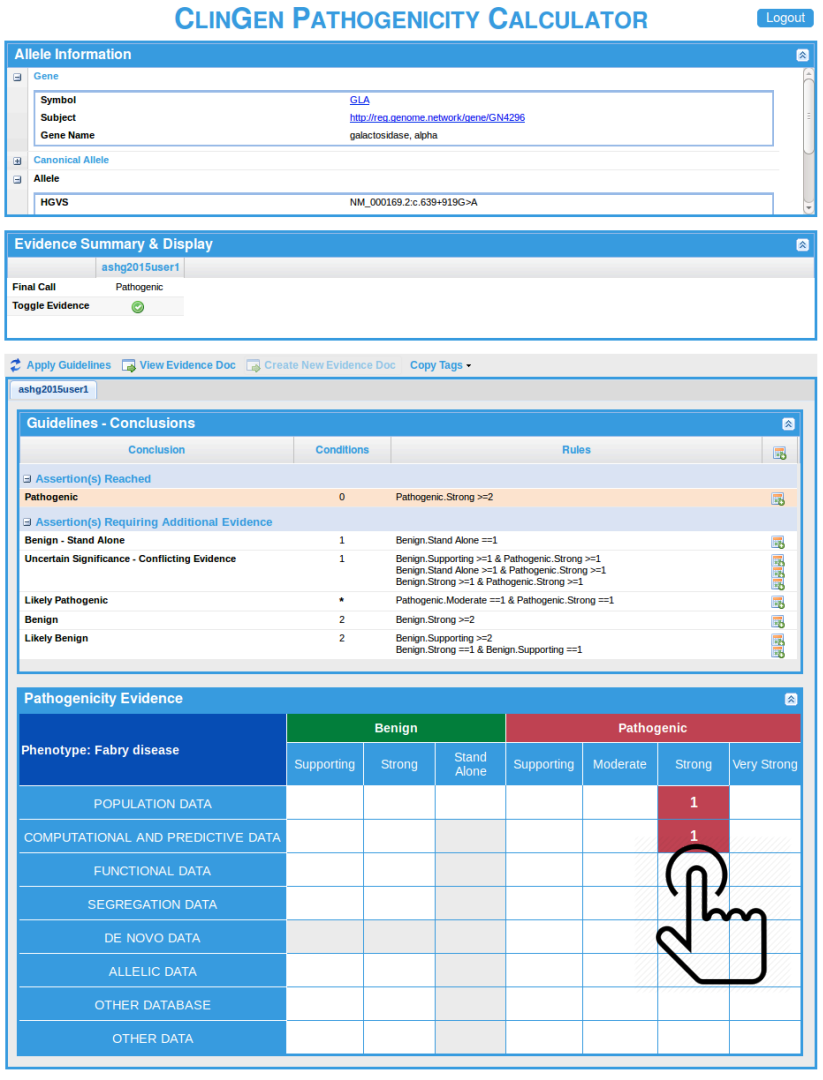


# Step 3: Create evidence document and input evidence: Turn PVS1-Strong tag ON

See slide #21 for details

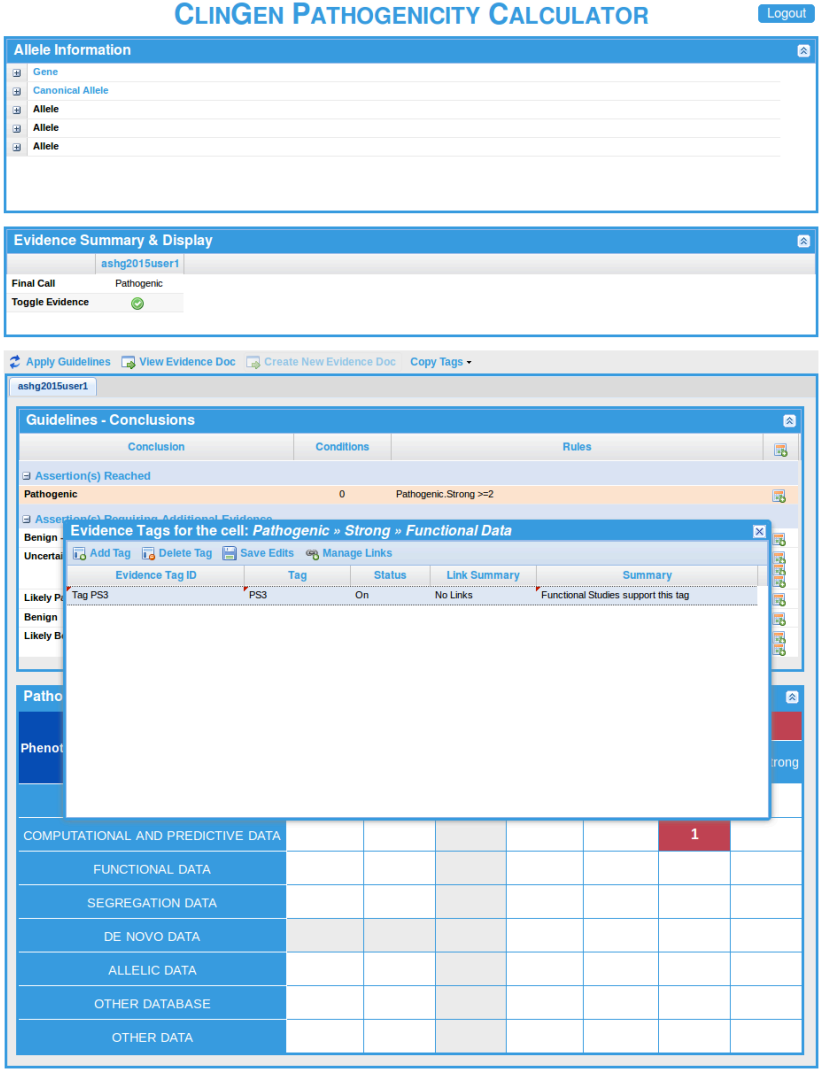


# Step 3: Create evidence document and input evidence: Turn PS3 tag ON

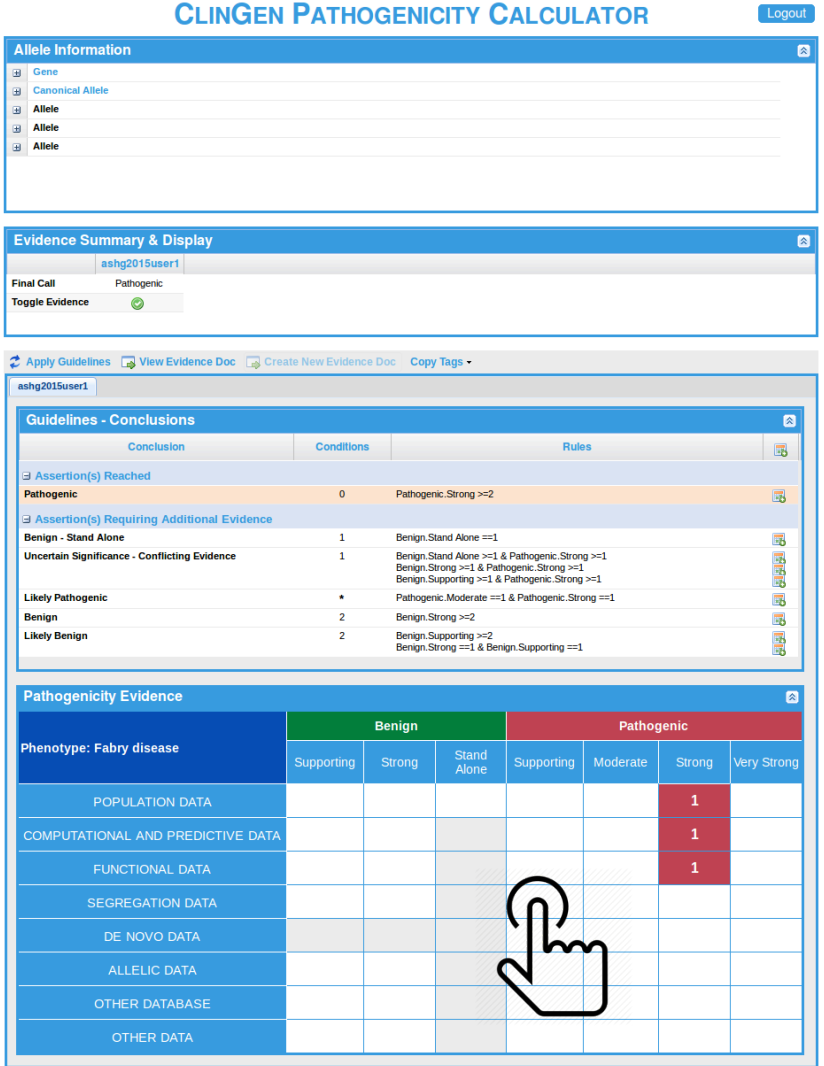


# Step 3: Create evidence document and input evidence: Turn PS3 tag ON

See slide #21 for details

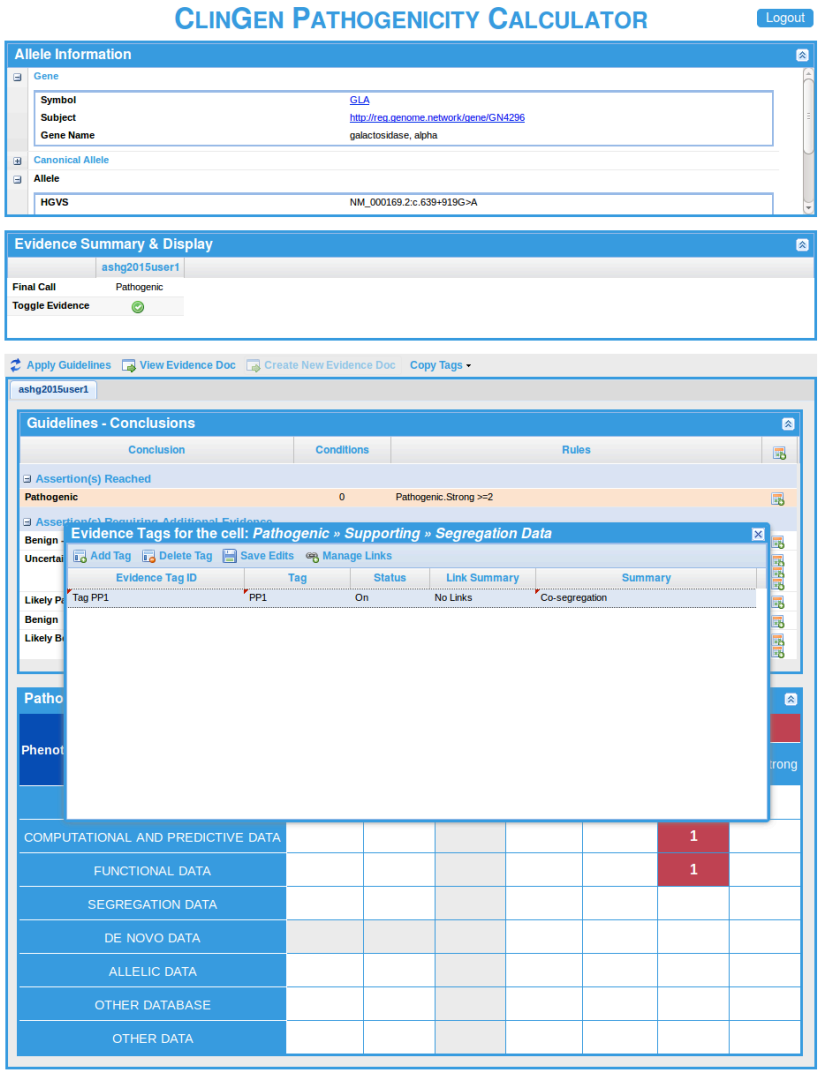


# Step 3: Create evidence document and input evidence: Turn PS3 tag ON



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

See slide #21 for details



## Conclusion and Reasoning

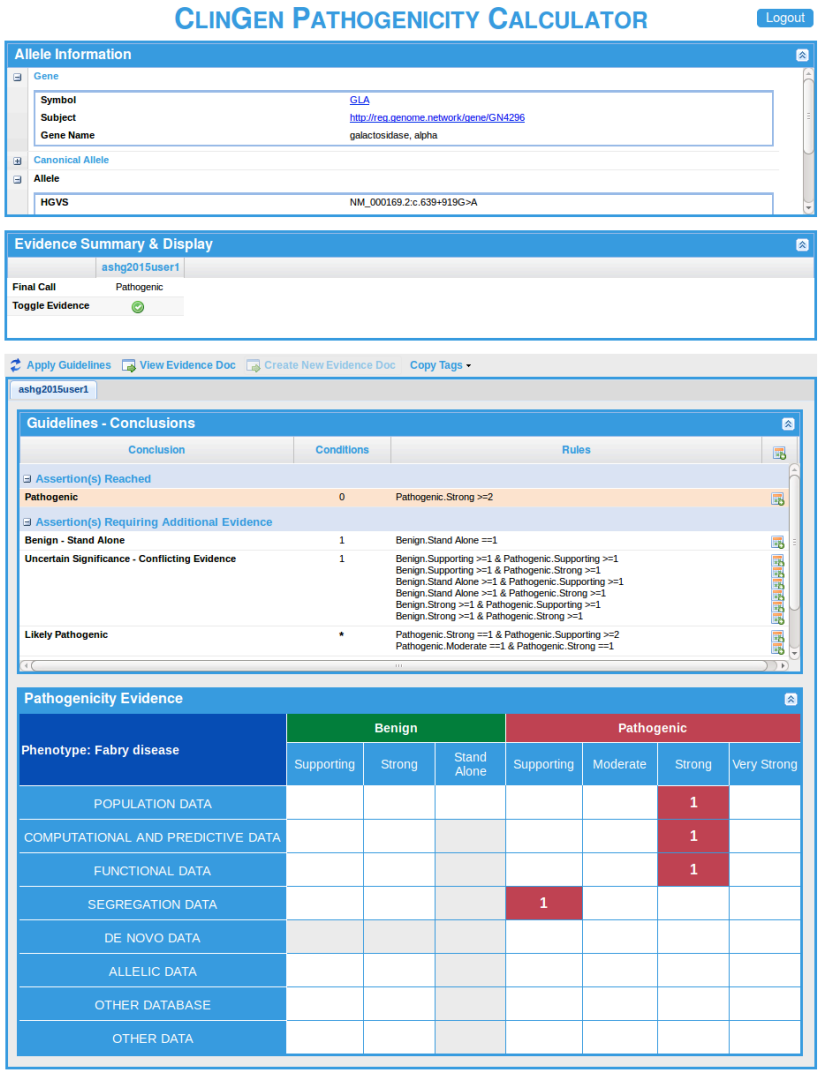
The conclusion reached is “Pathogenic”.

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.

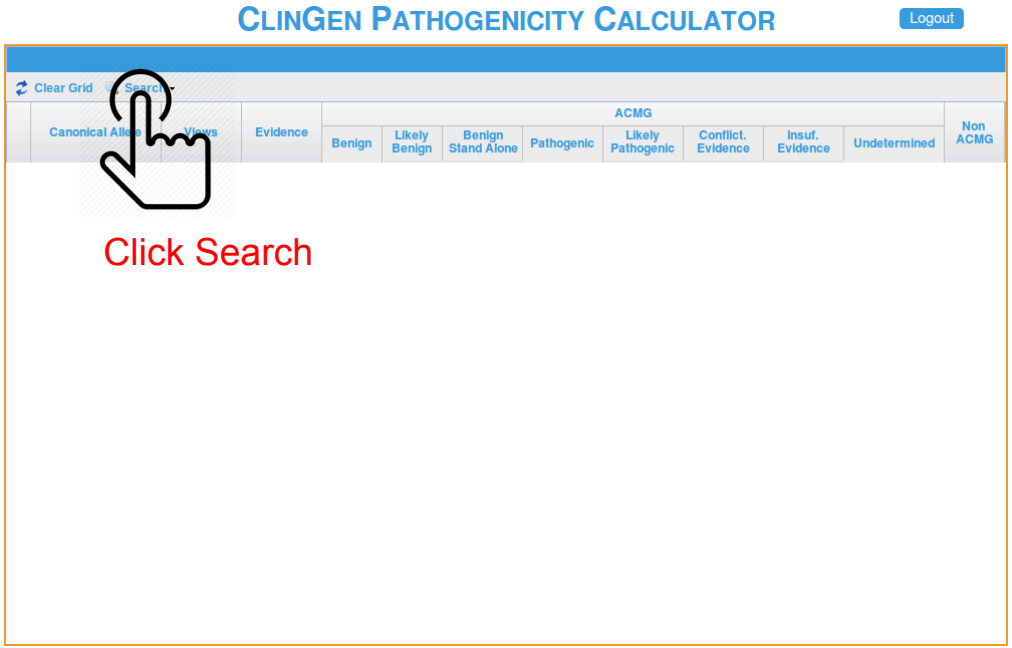
For each rule that is not satisfied, the number of missing evidence items is listed.

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.

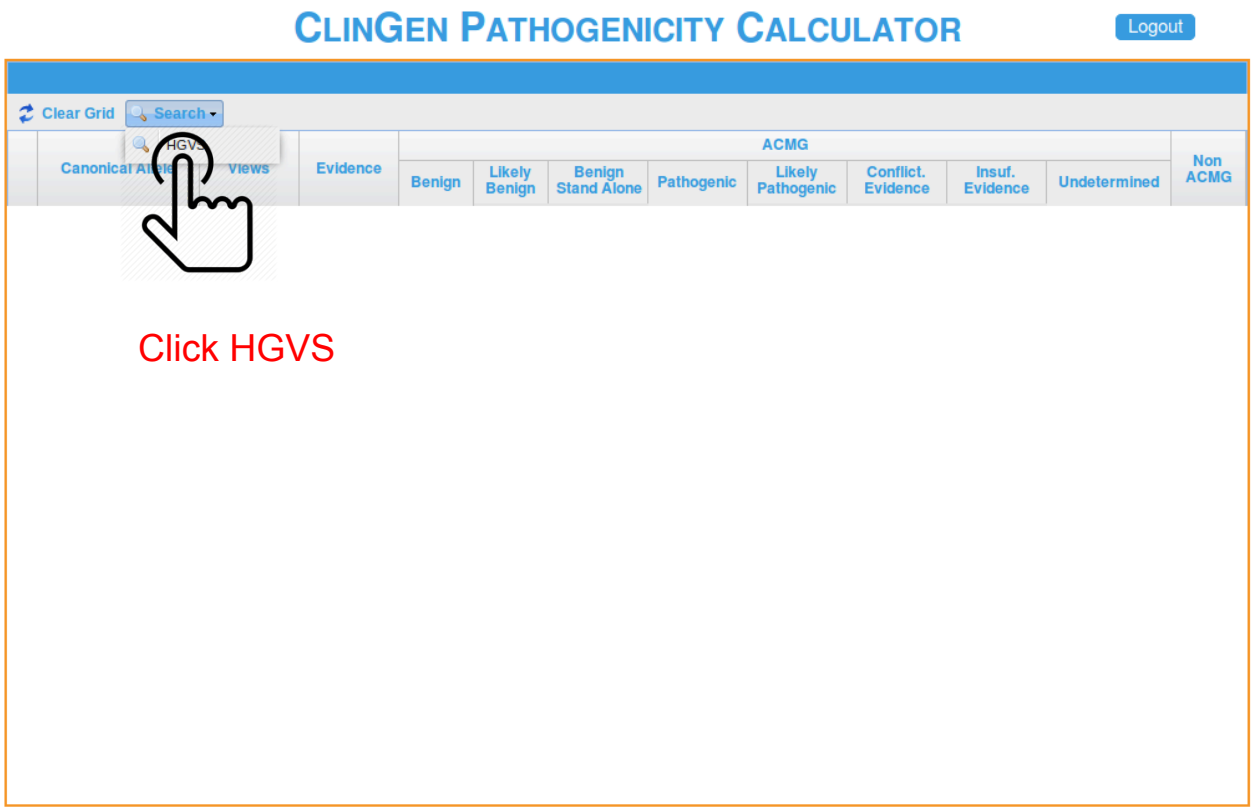


# Step 5: Retrieve stored evidence and conclusions: Activate HGVS based search

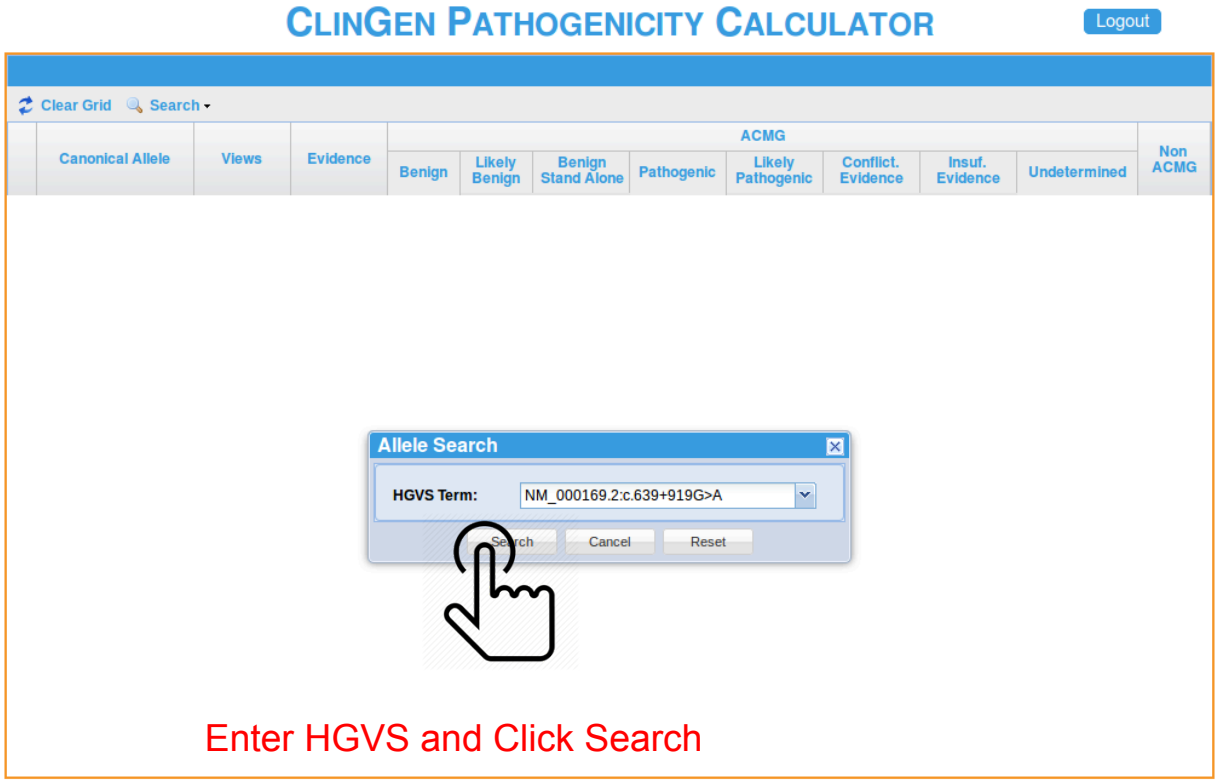
Visit: Perform the HGVS search for the same allele:
calculator.clinicalgenome.org/java-bin/clingenV2.0.jsp



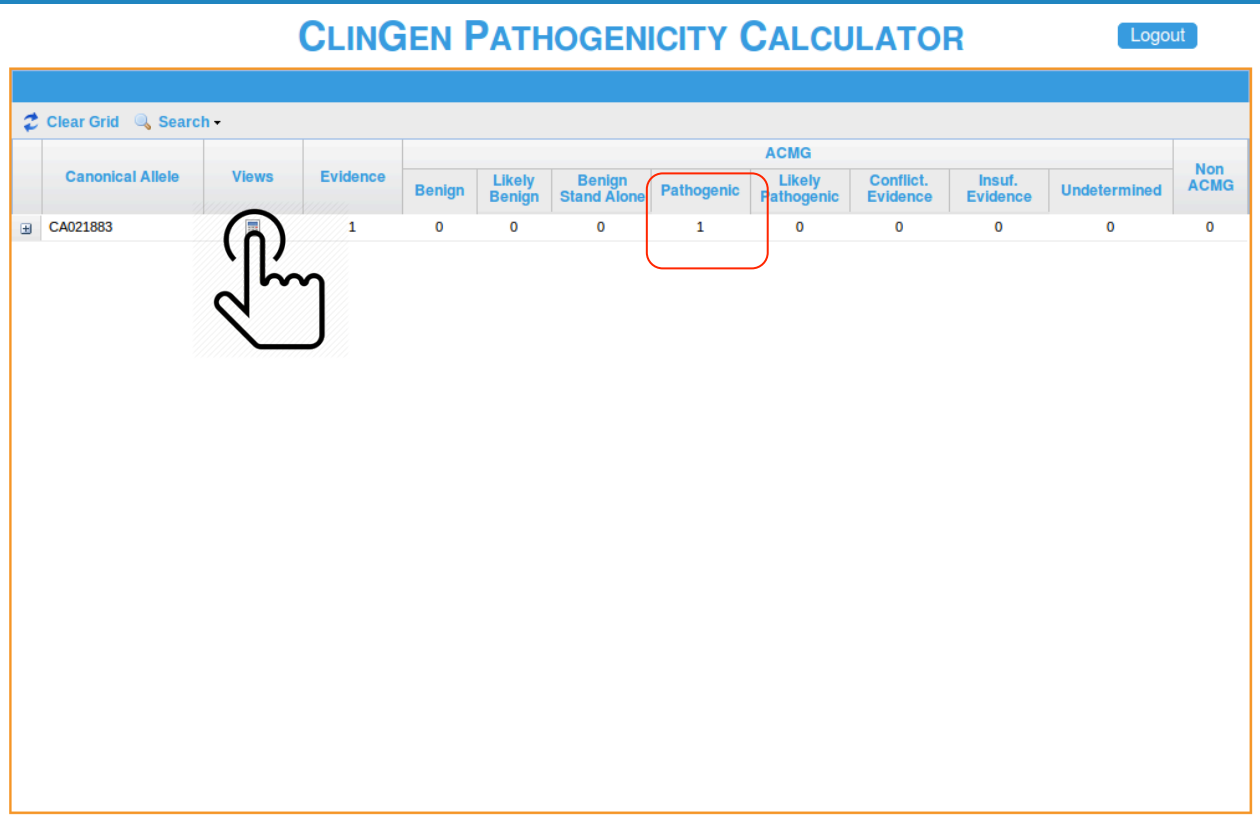
# Step 5: Retrieve stored evidence and conclusions: Activate HGVS based search



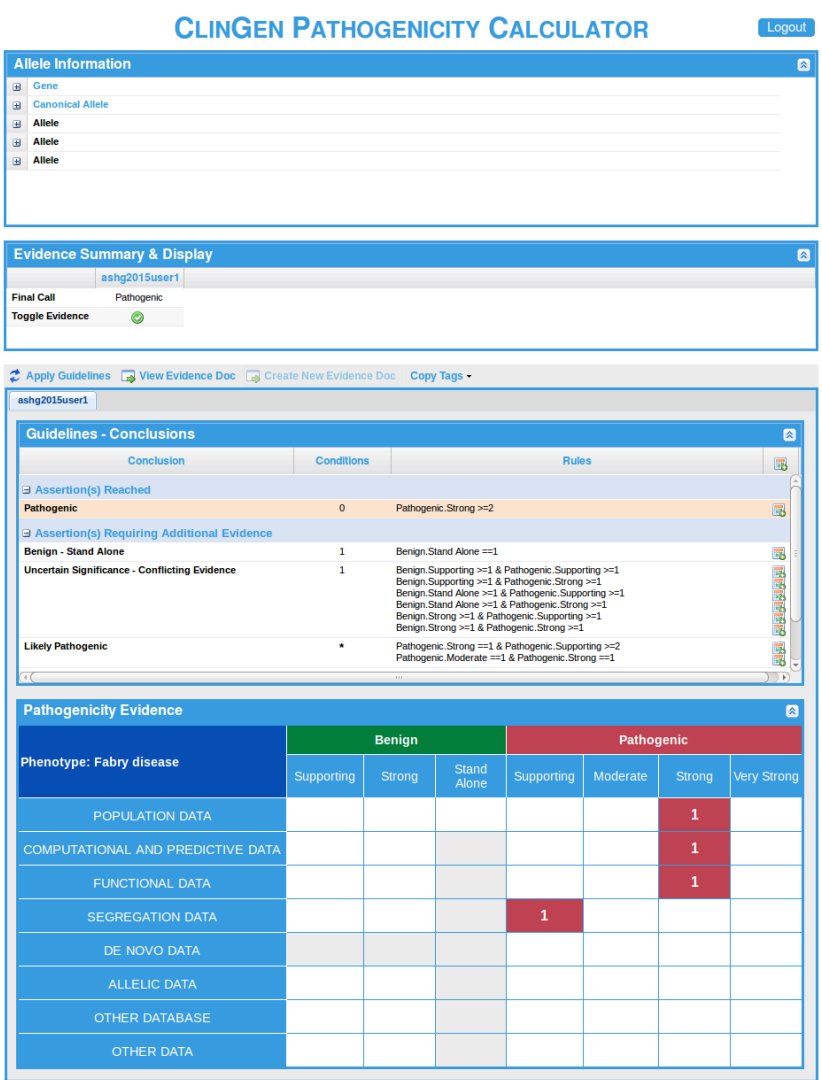
# Step 5: Retrieve stored evidence and conclusions: Search for NM_000169.2:c.639+919G>A



# 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

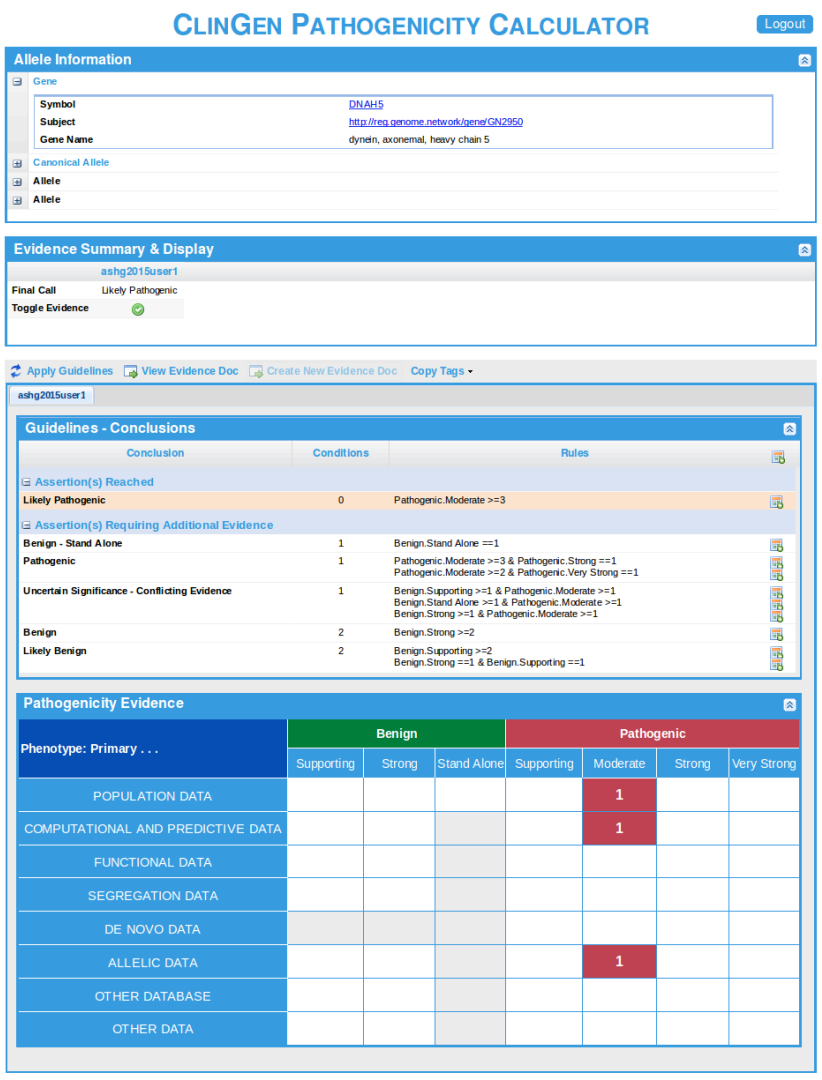
Turn the following evidence tags on:
PM2, PM3, PM4 for Primary ciliary dyskinesia

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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