



## **Standardizing ACMG Variant Interpretation Guidelines**

Gabe Rudy | VP of Product &  
Engineering

# Golden Helix – Who We Are



**Golden Helix is a global bioinformatics company founded in 1998.**



**Variant Calling**  
**Filtering and Annotation**  
**Variant Interpretation**  
**Clinical Reports**  
**CNV Analysis**  
**Pipeline: Run Workflows**

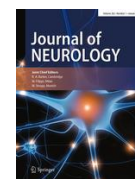
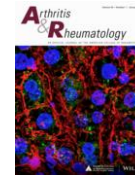
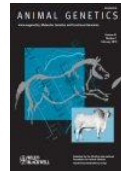
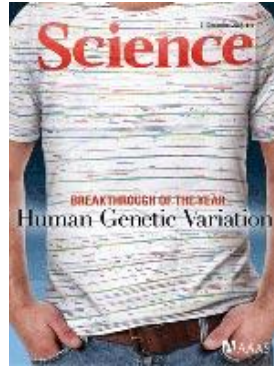


**Variant Warehouse**  
**Centralized Annotations**  
**Hosted Reports**  
**Sharing and Integration**

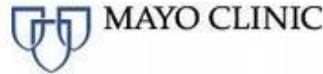


**CNV Analysis**  
**GWAS**  
**Genomic Prediction**  
**Large-N-Population Studies**  
**RNA-Seq**  
**Large-N CNV-Analysis**

Cited in over 1,200 peer-reviewed publications



Over 350 customers globally



# Golden Helix – Who We Are



When you choose a Golden Helix solution, you get more than just software

- REPUTATION
- TRUST
- EXPERIENCE



- INDUSTRY FOCUS
- THOUGHT LEADERSHIP
- COMMUNITY

- TRAINING
- SUPPORT
- RESPONSIVENESS



- INNOVATION and SPEED
- CUSTOMIZATIONS

# Genetic Testing Process



## Golden Helix Clinical Suite



Sample Prep



Sequencing



Align & Call



Annotate  
& Filter



Variant  
Interpretation



Report

**Sentieon  
& VS-CNV**

**VarSeq**

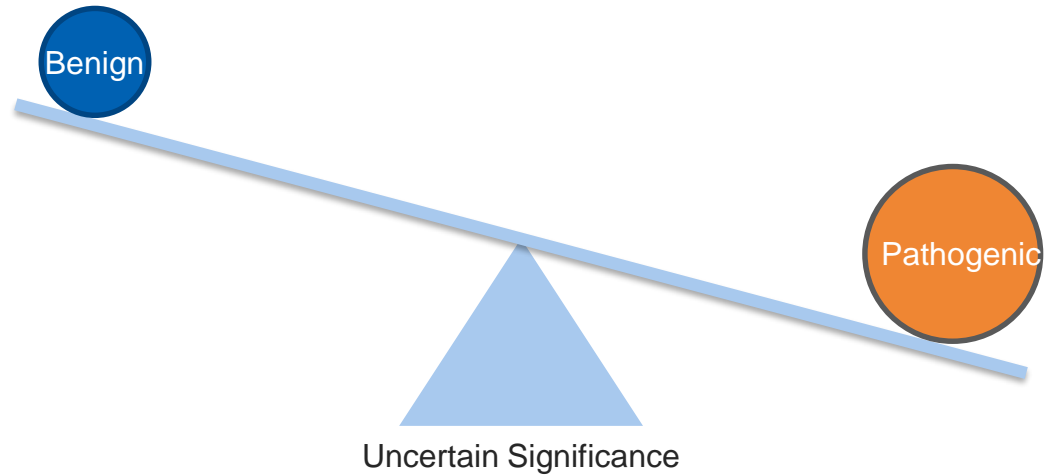
**VSClinical**

**VSReports**



## ■ Evaluation of Evidence:

- Clinical presentation
- Gene function
- Bioinformatic evidence
- Population frequencies



# ACMG Guidelines



- A standardized process for scoring variant for genes that cause Mendelian disorders.
- Widely adopted in US, and worldwide
- 33 criteria for evaluating a variants evidence
- Rules for combining criteria into a single Classification
- Not static, follow up papers and expanded recommendations published

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**ACMG STANDARDS AND GUIDELINES**

**Genetics  
in Medicine**

**SPECIAL ARTICLE**

**Genetics  
in Medicine**

**ORIGINAL RESEARCH ARTICLE**

**Genetics  
in Medicine**



## **ACGS Best Practice Guidelines for Variant Classification 2017**

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Recommendations ratified by ACGS Quality Subcommittee on 2<sup>nd</sup> October 2017

### **1. Introduction**





- **Complete Support for ACMG Guideline Workflow:**

- Implements a guided workflow for following the ACMG guideline scoring and classifying
- Place criteria into conceptually related groups, paired with their opposites, and formatted as answerable question.

- **Aggregate and Automate:**

- Questions have supporting evidence presented with rich and interactive visuals
- Automatically computed recommendations for questions that have explicit bioinformatic evidence, with supporting reasons for each answer.

- **Expert and Beginner Friendly:**

- Start with descriptive summaries and recommendations for a variant
- Deep dive into Population Catalogs, Gene Impact, Published Studies and Clinical tabs
- Integrated documentation, readings on scoring criteria and citations

# Analysis Workflow with VSClinical



- 1. Follow your existing VarSeq annotation and filtering workflow**
- 2. Add new ACMG Auto Classifier algorithm:**
  - Looks up if variant annotated in previous sample
  - Scores 18 criteria based on available evidence from 7 sources
- 3. Select variants to evaluate using the ACMG Guidelines**
- 4. Score and Finalize each variant, selecting which to report**
- 5. Finalize the sample, review and report**

The screenshot shows the 'Filter Chain' interface in VSClinical. It displays a list of filters applied to a set of variants, with the total number of variants remaining after each filter step.

Filter	Count
Filter Chain	961
Filter (Current) is PASS	730
Read Depths (DP) (Current) > 100	678
Clinical Significance is (Pathogenic, Uncertain Significance)	10
All MAF < 0.3 OR missing	8
Effect (Combined) is (LoF, Missense)	6
LoF	1
Missense	5
Other	2
Missing	0



[Demo in VarSeq]

# How Does VSClinical Work?



- **7 Algorithms and 5 Annotation Sources**
- **On the fly computation, thinner projects**
- **Exclusive to VS Clinical**
  - Splice Site Predictions
  - Multiple Sequence Alignment (MSA)
  - MSA derived GERP++ and phyloP
  - MSA derived SIFT and Polyphen2
  - ACMG Auto Classifier
- **New Annotation Sources**
  - ClinVar Assessments
  - Missense Badness

**PVS1** Null variant in a gene where LOF is a known mechanism of disease

Null variant types include nonsense, frameshift, canonical  $\pm 1$  or 2 splice sites, initiation codon, single or multiexon deletion

Yes

No

## Reasons for No:

- The variant is 48nt upstream of the penultimate coding exon junction, which is below the threshold estimated for nonsense-mediated decay to destroy the protein product.
- The variant overlaps two other transcripts in which it is not classified as a null variant.

## Reasons for Yes:

- The gene CFTR has a pLI score of 0.98, which is above the 0.9 threshold to indicate a gene that is intolerant of LoF indications.

# Golden Helix Pathogenicity Calculator



## ✓ ACMG Classification

### ■ Probabilistic Model of Pathogenicity:

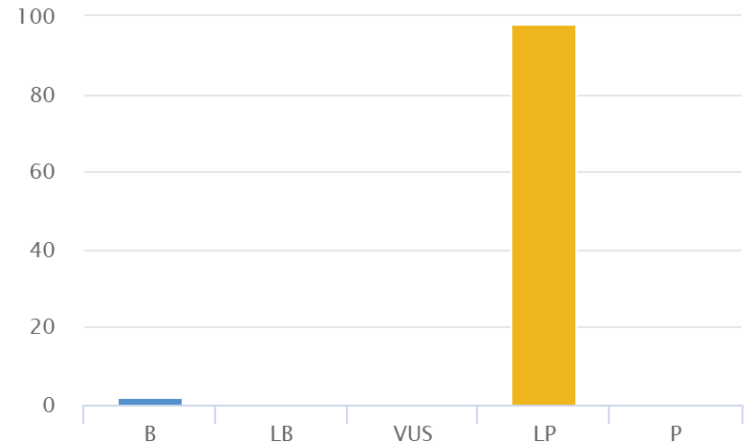
- Provide instant feedback
- Per classification probabilities
- Combined pathogenicity probability
- Necessary to classify a variant with conflicting criteria

Probability of Pathogenic given Scored Criteria:



Status: Probability of 0.93 is above 0.90 threshold for **Likely Pathogenic**

Probability for Each Classification:



# Transcript Aware Analysis



- Updated selection of clinically relevant transcript based on ClinVar Assessments
- Your transcript preferences saved and used in VarSeq + VSClinical
- Warning if the the variant overlaps other transcripts that have a different effect
- Can switch to view/interpret on any other transcript

## Overlapping Transcripts for CDKN2A





Transcript	Used in Interpretations	Effect	HGVS	Length CDS/AA	Exon Position	Protein ID / LRG ID	Coding Status
<a href="#">NM_058197.4</a>	Internal, ClinVar 2 Cases, 1 Assessment	Frameshift frameshift_variant	c.105dupG p.A36Gfs	351 bp 36 AA	1 of 3 30% of CDS	NP_478104.2	Complete
<a href="#">NM_001195132.1</a>	Internal, ClinVar 2 Cases, 1 Assessment	Frameshift frameshift_variant	c.105dupG p.A36Gfs	504 bp 36 AA	1 of 4 21% of CDS	NP_001182061.1	Complete
<a href="#">NM_000077.4</a> Default for Gene , Has LRG	Internal, ClinVar 2 Cases, 1 Assessment	Frameshift frameshift_variant	c.105dupG p.A36Gfs	471 bp 36 AA	1 of 3 22% of CDS	LRG_11t1 NP_000068.1	Complete Default
<a href="#">NM_058195.3</a> Has LRG	Internal, ClinVar 2 Cases, 1 Assessment	Intron intron_variant	c.194-3515dup G	399 bp false	2 of 3 false	LRG_11t2 NP_478102.2	Complete

# Functional Predictions



Functional Predictions: ⓘ

Primates **Mammals** Vertebrates

MSA-SIFT	Damaging		1.00 (greater than 0.95)
MSA-PolyPhen2	Damaging		0.909 (greater than 0.446)
PhyloP	Not Conserved		0.94 (less than 1.25)
GERP++	Conserved		4.23 (greater than 4)

Orangutan

## Functional Predictions:

- 100-way multiple-sequence alignment
- Run on Primates, Mammals, Vertebrates
- Algorithms run on-demand for any variant
- Can be run as a bulk annotation algorithm
- *Upcoming webcast will cover more*

### Multiple Sequence Alignment

100 Way Multi Species Alignment DNA AA

```
Human A CTA ACC CTT TCA GGT CTA AAT GGA GCC CAG ATG GAG AAA AT
Alt A 32,906,805 T 32,906,846
Chimp A CTA ACC CTT TCA GGT CTA AAT GGA GCC CAG ATG GAG AAA AT
Goril A CTA ACC CTT TCA GGT CTA AAT GGA GCC CAG ATG GAG AAA AT
Orang A CTG ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Gibbo A CTA ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Rhesu A CTA ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Crab- A CTA ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Baboo A CTA ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Green A CTA ACC CTT TCA GGT CTA AAT GGA ACC CAG ATG GAG AAA AT
Marmo A CTA ACC CTT TCC GGT CTA CAT GGA ACC CAG ATG GAG ATA AT
Squir - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Bushb T CTA ACC CTT TCA GGT CTA AGT GGA ACC CAG ATG GAG AAA AT
Chine G CTA AAG ATT TCA GAA CTG AAT AGA ACC CAG ATA GAG AAA AT
```

# Splice Site Predictions



Splice Predictions: ⓘ

GeneSplicer	Disrupted		0.00 (less than 0.59)
MaxEntScan	Disrupted		0.13 (less than 0.42)
NNSplice	Disrupted		0.28 (less than 0.85)
PWM	Not Disrupted		0.71 (greater than 0.66)

Nearest Splice Site:

Donor of Exon 5 of 16

Distance to Splice Site:

2bp upstream (Intronic)

## ■ Required for ACMG Classification:

- Variants near splice site, synonymous
- 4 algorithmic strategies
- Detect deletion of canonical splice site
- Run in VSClinical and as part of our gene annotation algorithm
- *Upcoming webcast to cover more*

## Multiple Sequence Alignment ⓘ

100 Way Multi Species Alignment

DNA AA

```
Human GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAAAAAA
Alt A 47,641,537 T 47,641,582
Chimp GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Goril GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAAAAAA
Orang GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAA-----
Gibbo GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Rhesu GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Crab- GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Baboo GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Green GA GCC CTT AAC CTT TTT CAG GTAAAAAAAAAAAAAAAAAAAAA-----
Marmo GA GCC CTT AAC CTT TTC CAG GTAAAAATAAAAATAAAA-AAAATA
Squir -----
Bushb GA GCC CTT AAC CTT TTC CAG GTAAACAAAAACAAATAA-----TA
Chine GA GCC CTT AAC CTA TTC CAG GTAAAAAAAAAAAACAAAAA-AAAACA
```



# Splice Region Analysis



### Splice Region for NM\_000059.3

Forward Strand (NM\_000059.3): ⓘ

Ref	TTGTTTATGCATCATGTTTCTTTAGAGCCGATTACCTGTGTACCCTTTCTGTAAGACATGTTTAAATTTTCTAAATTTCTAATACAGTATGAGAAAAG	
Pos	32,920,983	32,921,080
Alt	TTGTTTATGCATCATGTTTCTTTAGAGCCGATTACCTGTGTACCCTTTCTGTAAGACATGTTTAAATTTTCTAAATTTCTAATACAGTATGAGAAAAG	
Pos	32,920,983	32,921,080

GS Ref  
GS Alt

MES Ref  
MES Alt

NNS Ref  
NNS Alt

PWM Ref  
PWM Alt

Reverse Strand: ⓘ

Ref	CTTTTCTCATACTGTATTAGAATTTAGAAAAATTTAAACATGTCTTACCGAAAGGGTACACAGGTAATCGGCTCTAAAGAAACATGATGCATAAACAA	
Pos	32,921,080	32,920,983
Alt	CTTTTCTCATACTGTATTAGAATTTAGAAAAATTTAAACATGTCTTACAGAAAGGGTACACAGGTAATCGGCTCTAAAGAAACATGATGCATAAACAA	
Pos	32,921,080	32,920,983

GS Ref  
GS Alt

MES Ref  
MES Alt

NNS Ref  
NNS Alt

PWM Ref  
PWM Alt

Ok

29 Variants: 28 T

Variant:

GRCh37:  
chr13: 32,921,03

Genotype:  
Heterozygous

dbSNP:  
rs28897743

Gene:

Gene:  
*BRCA2*

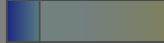
NM\_000059.3:  
c.7007G>T

Effect:  
Missense  
missense\_variant

ACMG Scoring

Scored Criteria:  
2 criteria currently unscor

Probability:



56.2% bayesian model prob

Classification:

Not Classified

Previously classified

# Standardize your Lab with VSClinical



- **Reduce Subjectivity / Errors**

- Supports "Blinded Analysis" of same sample by two lab personnel

- **Self-learning System**

- Knowledge of assessments
- Historical answers for same variant and similar variants in gene help inform decision

- **Scale up Interpretations**

- Self guided exploration of details
- Automate google searchers for variants in papers

## ACMG Guidelines based Evaluations of Variants

Gabe Rudy ACMG Guidelines V1  
2 days ago

**20 Variants**

Finalized: 1 Pathogenic, 2 Likely Pathogenic, 10 VUS  
⚠ Different than the 17 currently selected to interpret [Compare](#)

🔒 Finalized Reopen View

Nathan Fortier, Gabe Rudy ACMG Guidelines V1  
yesterday

**17 Variants**

Status: 10 To Review, 2 Finalized, 5 Previously Classified

Open and Continue

Nathan Fortier, Gabe Rudy ACMG Guidelines V1  
yesterday

**17 Variants**

Status: 10 To Review, 2 Finalized, 5 Previously Classified

Start New Blinded

# Thanks to NIH & Stakeholders



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- Dr. Ahmed Alfares, King Abdul Aziz Medical City, Saudi Arabia),
- Dr. Bailey Glen (Medical University of South Carolina, USA)
- Dr. Jim Weber (PreventionGenetics, USA)
- Dr. Qin Hae and Dr. Line Larsen (Amplexa, Denmark)
- Dr. Val Hyland (Pathology Queensland, Australia).

# Summary & Next Steps



- Contact us to evaluate!
  - New Product
  - Includes OMIM, CADD, VSReports
  - Splice site predictions
  - Functional predictions
- Part of upcoming VarSeq 2.0 release
- Shipping Q2, targeting end of May
- Contact us if you are interested in other workflows or customizations

