

# 生殖細胞系列バリエーションの病原性評価

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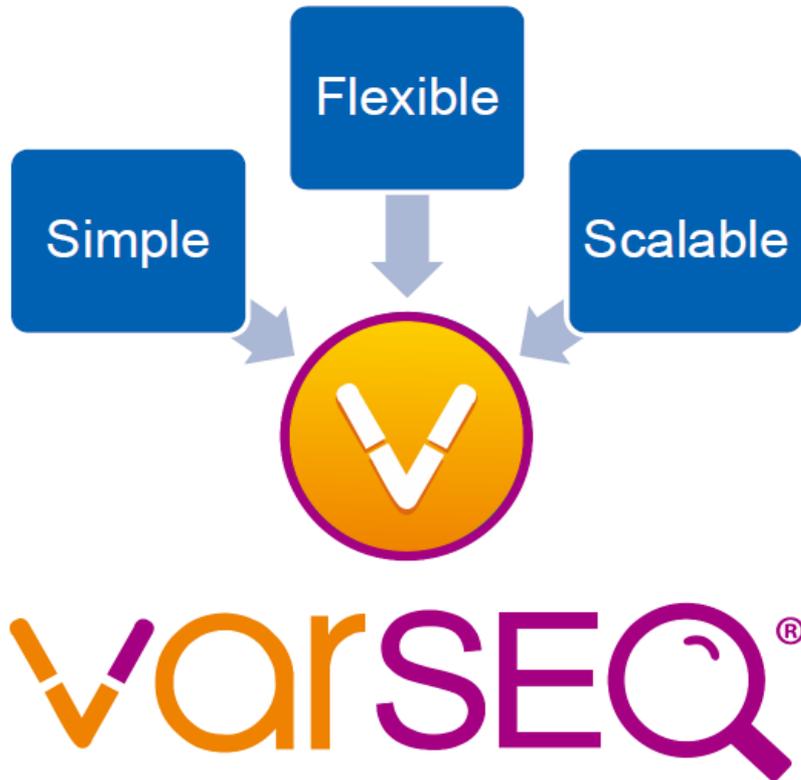
- 次世代シーケンサーでは膨大な遺伝子バリエーションが検出されるが、臨床的な意義が不明なものも多く、その病原性の評価には、文献やデータベース検索、複雑なバイオインフォマティクス解析などが必要

- Golden Helix社VarSeq®の有償アドオンであるVSClinical ACMGでは、ACMGガイドラインに基づき、生殖細胞系列バリエーションの病原性を自動で評価することで、遺伝学的検査の結果の解釈を、効率的に行うことが可能

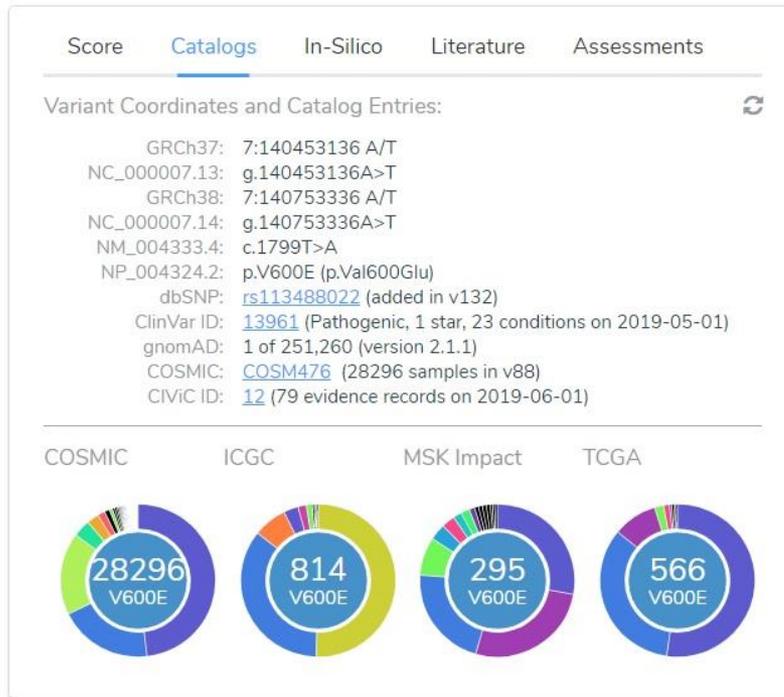
Richards et al.

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



- キュレーションされた様々なデータリソースを使用し、変異データへアノテーション付けを実行
  - RefSeq Genes
  - dbSNP
  - ClinVar
  - CIVic
  - ICGC / TCGA
  - PhrmaGKB
  - BRCA Exchange
  - 1000 Genomes
  - NHLBI 6500 Exomes
  - ExAC Variant
  - gnomAD Exomes/Genomes
  - dbNSFP
  - 各種遺伝子パネルのターゲットデータ
  - ...など
- VCFファイルに含まれる変異データから、任意の検索条件でデータのフィルタリングを行うワークフローを作成
- カバレッジ計算やトリオ解析、表現型情報に基づく遺伝子ランキングなどの解析アルゴリズムを搭載
- ゲノムブラウザーを搭載し、BAMファイルデータや各種アノテーションデータをグラフ表示
- 有償アドオンによる機能拡張で、CNVコールやレポート作成、パイプライン機能などが利用可能



Genotype: **Heterozygous**

Allele Ratio: 

dbSNP: **rs137854557**

ClinVar: **354**

Gene: 

Gene: **NF1**

Transcript: **NM\_000267.3**

NM\_000267.3: **c.1466A>G**

NP\_000258.1: **p.Y489C**

Effect: **Missense**  
missense\_variant

Exon: **13 of 57**

ACMG Scoring: <sup>2</sup> 

Scored Criteria:

**PP2 PP3 PS1**

2 criteria currently unscored

Probability of Pathogenic:



87.1% - Predicted Classification: Likely Pathogenic

Classification:

**Likely Pathogenic** 

- ACMG/AMPガイドラインに基づいた、遺伝子バリアントの自動評価
- 生殖細胞系列バリアントの病原性と、体細胞バリアントの腫瘍原性の評価に対応
- 評価結果および、疾患や治療薬情報などと合わせたレポート出力



## VS Clinical (ACMG)

- メンデル遺伝病における生殖細胞系列バリエントを、ACMGガイドラインの33種類の評価基準に基づいて分類し、病原性 (Pathogenic) や良性 (Benign) の判定を行う
- ガイドラインのうち18種類の評価基準については、バリエントのアレル頻度、機能予測、臨床情報データベースなどを用いて、自動分類を実行
- 専用の分類用ツールを実行することで、VCFファイルに含まれる全バリエントに対して一括で評価を行い、評価結果に基づきバリエントのフィルタリングが可能

## VS Clinical (AMP)

- がんにおける体細胞バリエントの腫瘍原性 (Oncogenicity) の評価に使用
- 体細胞バリエントのバイオマーカーとしての評価を、AMPガイドラインのEvidence tierレベルで分類し、治療薬や臨床試験情報を含めたレポートを作成
- 主要ながんにおけるバイオマーカー情報などを収録した、専用の知識ベースGolden Helix CancerKBが利用可能

## ■ VarSeq

- データリソースを利用した、バリエント（VCFファイル）へのアノテーション付け
- データリソースのアノテーションや疾患の遺伝形式などに基づいた、バリエントのフィルタリング
- ゲノムブラウザでのシーケンスデータ確認

## ■ VSClinical

- ACMGガイドラインに沿った、バリエントごとの病原性評価
- 遺伝子パネル実験のシーケンスカバレッジ確認
- レポート作成



Sample Prep

Sequencing

Align & Call

Annotate  
& Filter

Variant  
Interpretation

Report

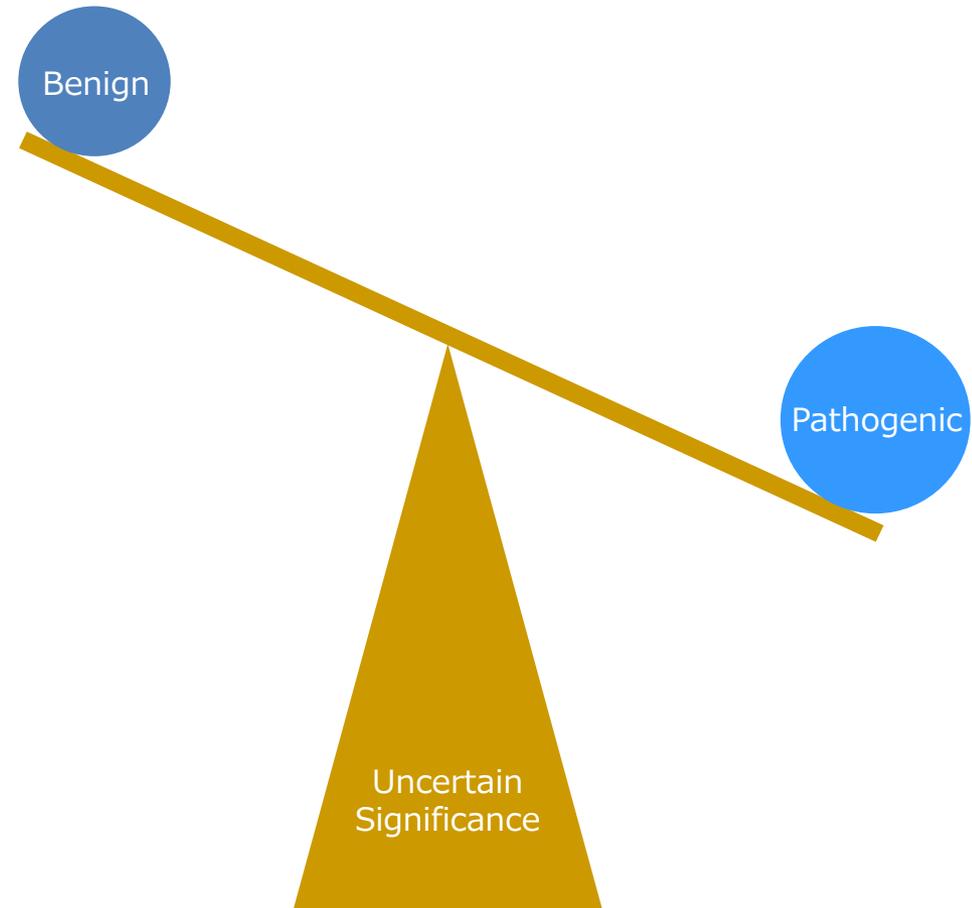
 varSEQ®  VS Clinical®

## ■ 病原性の評価方法

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

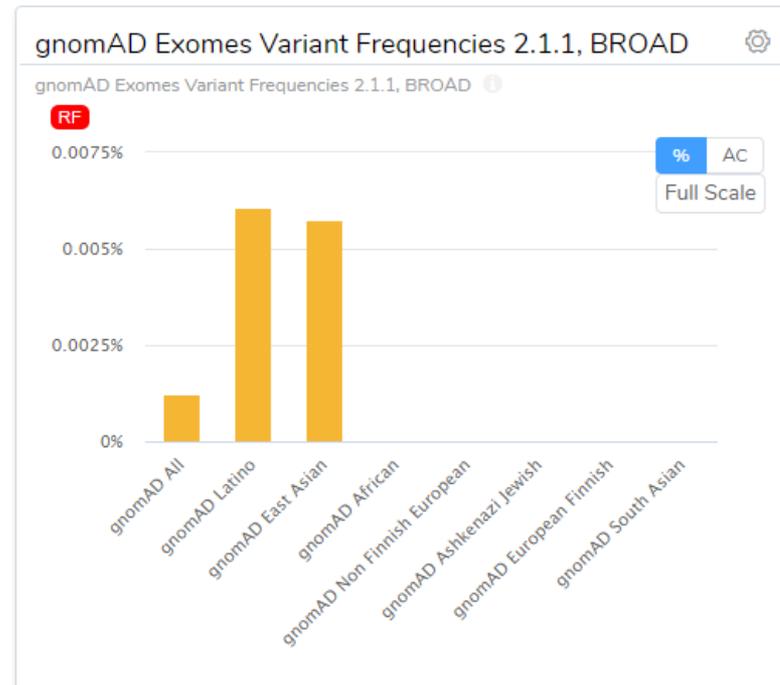
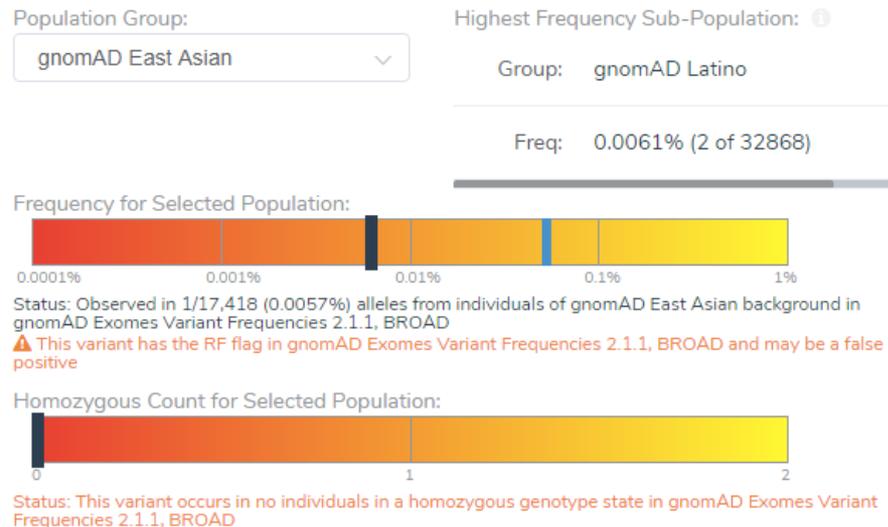
## ■ ACMGガイドライン

- 33種類の評価項目
- 評価スコアの合計より、バリエーションの病原性を5段階に分類



## ■ アレル頻度／コントロールデータベースを用いた評価

- gnomAD, 1000 Genomesおよびユーザーカスタムデータベースを使用
- 集団内のアレル頻度が高いバリエントは良性で、コントロールに存在しないか、または頻度の低いものは病原性とする
- データベース内の集団グループごとの頻度、Homozygous/Heterozygousごとの頻度なども確認可能



## ■ 遺伝子上の位置情報を用いた評価

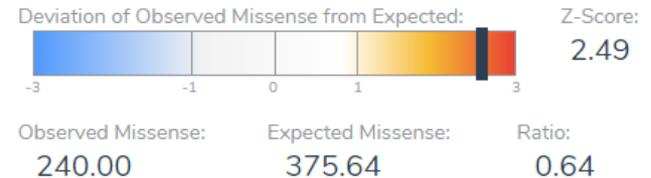
- バリエントが、遺伝子のホットスポットや機能ドメイン上に存在することによる評価
- ホットスポット上に存在し、なおかつ同じ領域に良性のバリエントが他に存在しない場合、病原性ありとする
- 評価画面上で、近傍領域のバリエント情報を確認

Variant Type:		Within Same:		Classification:		Rating:			
All	Missense	LoF	Gene	Exon	Codon	All	Benign	Pathogenic	<input checked="" type="checkbox"/> ☆ ☆ ☆ ☆ ☆
Dist AA/bp	Variant	Exon	Effect	Sources	Clinical Significance	Rating/Last Seen			
-1 / -4	c.766A>G (p.R256G)	7 / 18	Missense	ClinVar	Likely pathogenic	★ ★ ☆ ☆			
-1 / -3	c.767G>C (p.R256T)	7 / 18	Missense	ClinVar	Uncertain Significance	☆ ☆ ☆ ☆			
-1 / -2	c.768G>C (p.R256S)	7 / 18	Missense	ClinVar	Likely pathogenic	★ ☆ ☆ ☆			
-1 / -2	c.768G>T (p.R256S)	7 / 18	Missense	ClinVar	Pathogenic	★ ★ ★ ☆			
0 / -1	c.769T>A (p.S257T)	7 / 18	Missense	ClinVar	Likely pathogenic	★ ☆ ☆ ☆			
0 / -1	c.769T>C (p.S257P)	7 / 18	Missense	ClinVar	Likely pathogenic	★ ★ ★ ☆			
0 / 0	c.770C>G (p.S257W)	7 / 18	Missense	ClinVar	Uncertain Significance	★ ☆ ☆ ☆			
0 / 0	c.770C>T (p.S257L)	7 / 18	Missense	ClinVar	Pathogenic	★ ★ ★ ☆			
1 / 3	c.773C>G (p.T258R)	7 / 18	Missense	ClinVar	Likely pathogenic	★ ☆ ☆ ☆			
2 / 5	c.775T>A (p.S259T)	7 / 18	Missense	ClinVar	Pathogenic	★ ★ ★ ☆			

## ■ ミスセンスバリエーションに関する評価

- 評価対象の遺伝子において、ミスセンスバリエーションが疾患の原因となりうるかの評価
- データベース登録されている、良性のミスセンスバリエーションの割合や、コンピュータ予測によって、ミスセンスバリエーションの病原性を評価したスコアが計算される
- 良性のミスセンスバリエーションが多く、なおかつタンパク質の短縮を引き起こすバリエーションが疾患の原因だと考えられる場合は、ミスセンスバリエーションは良性と判定される

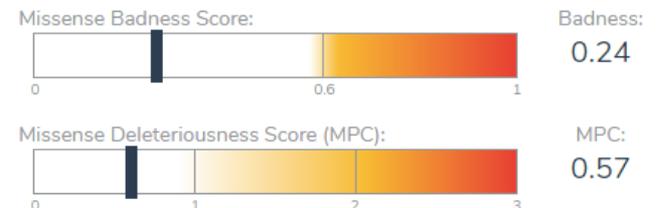
### Missense Mutation Rate for *RAF1*



Status: Low rate of missense variation (Z-Score > 1)

The gnomAD project has computed per-transcript counts of the number of missense variants per gene as an observed / expected score. The signed Z-score is the deviation of the observed counts from expected counts. Positive Z scores indicate increased constraint (intolerance to variation) and therefore that the gene had fewer missense variants than expected. See the [gnomAD v2.1 blog post](#) for more details.

### Missense Badness and MPC for c.770C>T (*RAF1*)

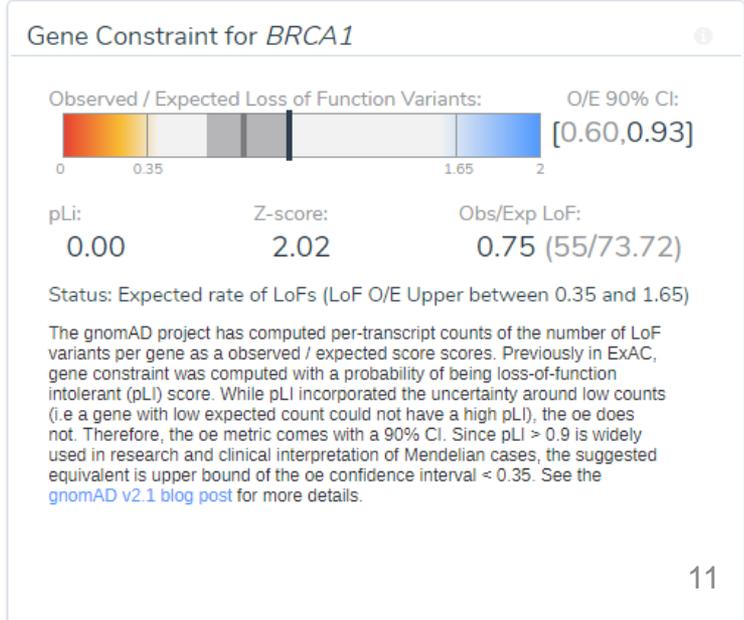
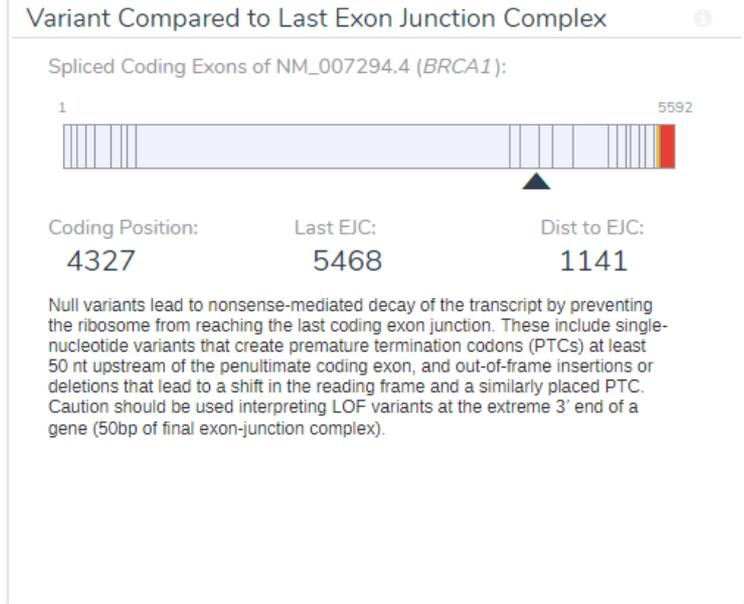


Status: Predicted to be a tolerated missense variant (MPC < 2)

Computed on an analysis of the ExAC population frequencies, the Missense Badness Score is the normalized fold difference of observed versus expected missense substitutions in sub-genic regions. This score is then combined with orthogonal deleteriousness metrics into one score called MPC (for Missense badness, PolyPhen-2, and Constraint) designed to classify whether a missense variant is deleterious. (DOI: [10.1101/148353](https://doi.org/10.1101/148353))

## ■ ナンセンスバリエーションに関する評価

- 評価対象の遺伝子において、終止コドンへの置換を引き起こす機能喪失 (LoF) バリエーションや、フレームシフトを引き起こすバリエーションが疾患の原因となりうるかの評価
- 同じ遺伝子上で、評価対象のバリエーションより下流に、病原性のナンセンスバリエーションが多数報告されている場合は、強い病原性をもつと判定されるが、バリエーションが遺伝子の最後のエクソン (または最後から2番目のエクソンの最終50bp以内) に位置する場合は、病原性なしとなる
- 遺伝子ごとに、ナンセンスバリエーションの実測値/期待値のスコアが計算されるので、この値に基づいて評価を行うことも可能



## ■ コンピュータ予測を用いた評価

- 生物種間の保存度や、SIFT, PolyPhen2などの複数のコンピュータ予測用ツールによって、バリエーションの有害性を判定する
- 同義バリエーションかつ、スプライシング異常を引き起こすものについては、GeneSplicerなどのスプライス部位予測用ツールが使用される

### Multiple Sequence Alignment

100 Way Multi Species Alignment Forward **Reverse** DNA AA

```

Human CC CTC TCC CAG AGG CAG AGG TCG ACA TCC ACA CCT AAT GTC C
Alt A 12,645,720 T 12,645,679
Chimp CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Goril CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Orang CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Gibbo CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Rhesu CC CTT TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Crab- CC CTT TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Baboo CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Green CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAC GTC C
Marmo CC CTC TCC CAG AGG CAG AGG TCA ACG TCC ACA CCT AAT GTC C
Squir CC CTC TCC CAG AGG CAG AGG TCG ACG TCT ACA CCT AAT GTC C
Bushb CC CTC TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Chine CC CTG TCC CAG AGG CAG AGG TCG ACG TCC ACA CCT AAT GTC C
Squir CC CTC TCC CAG AGG CAG ACG TCG ACA TCC ACA CCT AAT GTC C
    
```

### Functional Predictions: ⓘ

	Primates	Mammals	Vertebrates	
MSA-SIFT			<b>Damaging</b>	1.00 (greater than 0.95)
MSA-PolyPhen2			<b>Damaging</b>	1.000 (greater than 0.446)
PhyloP			<b>Conserved</b>	9.81 (greater than 2)
GERP++			<b>Conserved</b>	19.50 (greater than 10)

### Combined Annotation Dependent Depletion (CADD) Score: ⓘ

CADD	Uncertain	3.22 (between 2 and 5)
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### Disrupting Nearby Splice Predictions: ⓘ

GeneSplicer	<b>Disrupted</b>	0.59 (delta -0.40)
MaxEntScan	<b>Disrupted</b>	0.01 (delta -0.94)
NNSplice	<b>Disrupted</b>	0.32 (delta -0.60)
PWM	<b>Disrupted</b>	0.59 (delta -0.12)

## ■ 既報の臨床研究に基づいた評価

- すでに同じバリエーションが、データベース登録されていることに基づいた評価
- データベースで病原性バリエーションと登録されているものと同じアミノ酸置換を引き起こすものや、遺伝子上の同じ位置に存在し、異なるアミノ酸置換を引き起こすものに対する評価が可能
- 評価画面上で、データベース登録されているレコード情報や、出典論文情報なども確認が可能

### ClinVar Assessment For This Variant

◀ 1 of 20 ▶

HGVS: NM\_002880.3:c.770C>T  
 Classification: **Pathogenic** Date: 2020-01-13  
 Guidelines: Invitae Variant Classification Sherlock (09022015)  
 Disease: Noonan Syndrome 5; Leopard Syndrome 2; Noonan Syndrome with Multiple Lentiginos; Rasopathy; Noonan Syndrome; Not Provided; Lung Adenocarcinoma; Adenocarcinoma of Stomach; Malignant Melanoma of Skin; Neoplasm of the Large Intestine; Cardiomyopathy, Dilated, 1nn;leopard Syndrome 2;noonan Syndrome 5; Inborn Genetic Diseases; Noonan Syndrome;noonan Syndrome with Multiple Lentiginos; Noonan Syndrome 1  
 Source: Invitae

This sequence change replaces serine with leucine at codon 257 of the RAF1 protein (p.Ser257Leu). The serine residue is highly conserved and there is a large physicochemical difference between serine and leucine. This variant is not present in population databases (rs80338796, ExAC no frequency). This variant has been reported in many individuals affected with Noonan syndrome, both with and without multiple lentiginos (PMID: 17603482, 17603483, 20052757, 22389993). This variant was confirmed to be de novo in multiple affected individuals (PMID: 17603483, 23877478). ClinVar contains an entry for this variant (Variation ID: 13957). Experimental studies have shown that this missense change leads to increased activation of MEK, ERK, and ELK in vitro (PMID: 17603482, 20052757). For these reasons, this variant has been classified as Pathogenic. ⓘ

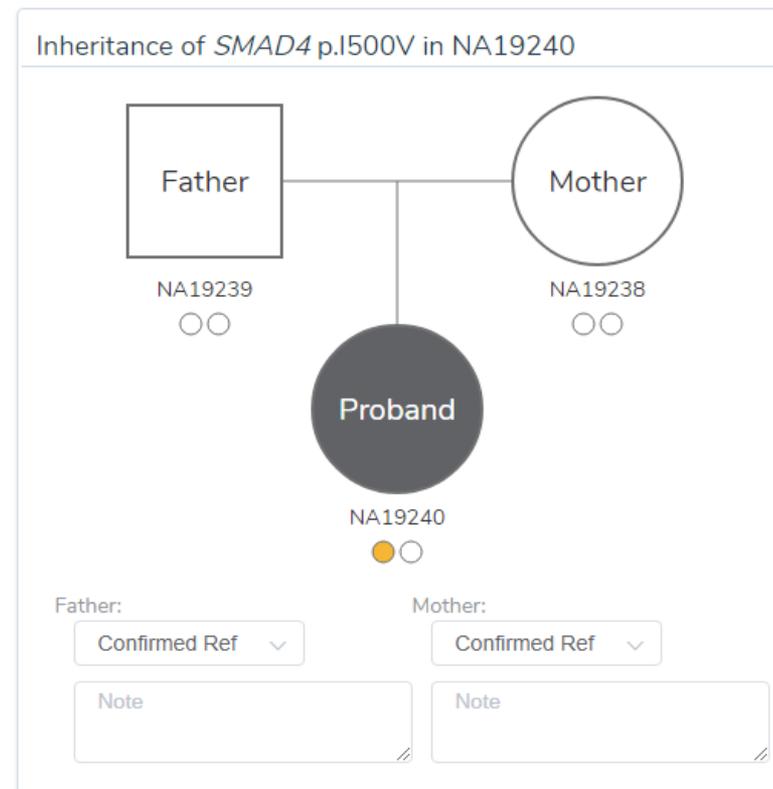
#### References:

- 20052757 ⓘ Molecular and clinical analysis of RAF1 in Noonan syndrome and related disorders: dephosphorylation of serine 259 as the essential...
- 22389993 ⓘ Two cases of LEOPARD syndrome--RAF1 mutations firstly described in children. Kuburović V et al. 53(6):687-91.
- 17603483 ⓘ Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. Pandit B et al....
- 17603482 ⓘ Germline gain-of-function mutations in RAF1 cause Noonan syndrome. Razzaque MA et al. 39(8):1013-7.
- 23877478 ⓘ Unique cerebrovascular anomalies in Noonan syndrome with RAF1 mutation. Zarate YA et al. 29(8):NP13-7.

Source: ClinVar (2020-01-13) ⓘ

## ■ 両親のサンプルデータを利用した評価

- 罹患者のサンプルに加え、その両親のサンプルのバリエーションデータを用いた評価
- 両親には存在せず、罹患者のみに存在するバリエーション（de Novoバリエーション）の場合は、病原性ありと判定される
- 両親がバリエーションをもたないことを確認済みの場合は、病原性が強いと判定されるが、未確認の場合は中程度の判定となる



## ■ 最終評価結果

- クリアした評価項目のID、および病原性の分類結果が表示される
- 各評価項目の強さや、サマリーなども確認が可能

Scoring System:

ACMG Variant Classification (Richards et al. 2015) [🔗](#)

To change the classification system close the evaluation and edit the Project Options...

Scored Criteria by Strength:

Pathogenic	Very Strong		x0
	Strong	PS1	x1
	Moderate	PM2, PM1, PM5	x3
	Supporting	PP2, PP3	x2
Benign	Supporting		x0
	Strong		x0
	Stand Alone		x0

ACMG Classification:

**Pathogenic**

Rule Pathogenic (iii): 1 Strong AND  $\geq 3$  Moderate, OR 2 Moderate AND  $\geq 2$  Supporting, OR 1 Moderate AND  $\geq 4$  Supporting

Recommended Criteria:

- Perform functional assay to determine the effect of the variant in the gene
- Establish the state of the variant in the parents

> ACMG Criteria Summary

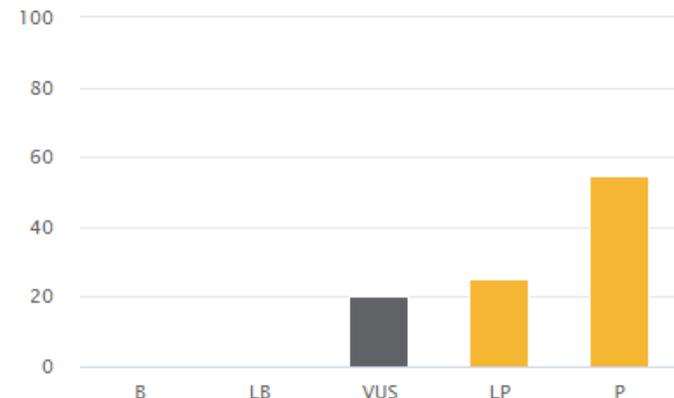
## Golden Helix Classification Prediction

Probability of Pathogenic given Scored Criteria:



Status: Probability of 88.7% predicting classification of **Pathogenic**

Probability for Each Classification:



A probability model was developed that takes the number of criteria scored at each evidence level and computes a probability of each of the five classifications. It was modeled and trained to agree with ACMG classification rules when provided non-conflicting criteria.

Scoring Annotations Gene Literature Assessments

Classification: **Pathogenic**      Scored Criteria: PM2 PM1 PP2 PP3 PS1 PM5

Evidence for Pathogenic: Interpretation Evidence Comments

The missense variant NM\_001354689.3(RAF1):c.770C>T (p.Ser257Leu) causes the same amino acid change as a previously established pathogenic variant. The p.Ser257Leu variant is novel (not in any individuals) in gnomAD All. The p.Ser257Leu variant is novel (not in any individuals) in 1kG All. There is a large physicochemical difference between serine and leucine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. The gene RAF1 has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 2.46. The gene RAF1 contains 40 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. 25 variants within 6 amino acid positions of the variant p.Ser257Leu have been shown to be pathogenic, while none have been shown to be benign. The p.Ser257Leu missense variant is predicted to be damaging by both SIFT and PolyPhen2. The serine residue at codon 257 of RAF1 is conserved in all mammalian species. The nucleotide c.770 in RAF1 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Pathogenic. ⓘ

[Add to Interpretation](#)



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Enterprise Blvd. Bozeman, MT  
Phone: (406) 867-3309 / Fax: (406) 555-6666  
http://goldenlabs.org/tests  
Accession ID: B124FC

Name: Jane Denver

DOB: 11/12/1965      MRN: MRN1234      Panel Coverage: 100.00      Date of Collection: 11/03/2020  
Sex: Unknown      Referring Facility: Enterprise Labs      Avg. Read Depth: 586.25x      Date of Receipt: 11/10/2020  
Family #:      Referring physician: Dr. Leonard McCoy      Specimen:      Date of Report: 12/01/2020  
Copies to:

Test(s) Performed: Targeted Gene Panel Sequencing  
Indication for test: Family history of ovarian cancer

**RESULT: Positive**  
Findings explain patient phenotype

**APPROACH**  
Sequencing of select genes was done using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. A total of N genes with previous implications in various mendelian disorders (see Supplement for a list of genes and coverage information) were covered with minimum read depth of 30X. Note that this test cannot exclude the possibility of variants in genes not analyzed or assayed with incomplete coverage.

**VARIANTS RELEVANT TO INDICATION FOR TESTING**  
One pathogenic variant in PTEN was identified in this individual. This individual has one CNV effecting genes associated with a disorder that is related to this individual's reported phenotype. No other variants of relevance to the indication were identified. Please see below for more detailed variant information.

Gene & Transcript	Variant	Allele State	Location	Disorder	Inheritance	Classification
PTEN NM_000314.8	p.His272Gln	Heterozygous	Exon 8	Prostate Cancer	Dominant	Pathogenic

**Copy Number Variations**

Name	Type	Size	Classification
BRCA2 ex17-19 dup	Duplication	8.0 kb	Likely pathogenic

**OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)**  
Incidental findings are variants of medical significance that are not associated with the individual's reported indication. Please note that the presence of pathogenic variants in genes with incomplete coverage or in genes not examined cannot be fully excluded.

**Carrier Status**  
This individual is a carrier of one heterozygous pathogenic variant in a gene associated with a recessive disorder that is unrelated to this individual's reported phenotype. In the heterozygous state, this variant is not known to play a role in disease. Please see below for more detailed variant information.

Signed (Automatically by DRAFT | Medical Director: Leonard McCoy, M.D. | CLIA Number: 22140803) (Not Signed Off)

## ■ バリアントの臨床的解釈とレポート

- 評価が完了すると、クリアした評価項目に基づき、バリアントの臨床的解釈のテキストが自動生成される
- 解釈のテキストは、サンプル情報や疾患情報などとともに、レポート出力が可能

## ■ 自動分類用ツールから実行する場合（ACMG Sample Classifier）

- 全バリエント（VCFファイル）へ、評価結果と関連データを自動でアノテーション付け
- レポート作成は不可

Chr:Pos	Ref/Alt	Zygoty	DP	Gene Name	HGVS pDot	Sequence Ontology	Classification	ACMG Classification Criteria
7:107824678	A/G	Homozygous Variant	2	NRCAM	?	splice_region_variant	Benign	BA1,BS2,BP4,BP7
7:117144425	-/A	Heterozygous	15	CFTR	?	splice_region_variant	Likely Benign	PM2,BP4,BP7
7:117176569	GATT/-	Heterozygous	4	CFTR	?	splice_region_variant	Benign	BA1,BP7,BP6
7:117180144	T/-	Heterozygous	5	CFTR	?	splice_region_variant	Likely Benign	PM2,BP4,BP7
7:117199533	G/A	Homozygous Variant	27	CFTR	NP_000483.3:p.Val470Met	missense_variant	Benign	BA1,BS2,BP6
7:117227860	G/A	Heterozygous	12	CFTR	NP_000483.3:p.Gly551Asp	missense_variant	Likely Pathogenic	PM1,PS1,PM5,PP3
7:117242922	G/-	Heterozygous	8	CFTR	?	splice_region_variant	Pathogenic	PM2,PVS1,PP3

## ■ グラフィカル画面でバリエントごとに評価を行う場合（Variant Dashbord）

- バリエントフィルタリングによって抽出された、少数のバリエントのみに対して、評価項目に関する質問の回答を選択していくことで評価を行う
- 推奨される回答は、バリエントデータに基づき、ソフトウェアが自動的にマーク付けする
- 推奨される回答の根拠も、画面上で確認が可能

**PS1** ⓪

Same amino acid change as a previously established pathogenic variant

The DNA level nucleotide change may differ, but result in the same protein level amino acid change

Yes
  No
  Uncertain

---

**Reasons for Yes:**

- The p.Ser257Leu variant is a missense mutation resulting in an amino acid change which is shared by the previously classified pathogenic variant p.S257L.

**Reasons for No:**

- The evidence used to form the basis for previous classification should be reviewed.

## ■ 自動分類用ツールから実行する場合

- 自動分類用ツール（ACMG Sample Classifier）では、全バリエントに対しての一括評価が可能
- 評価に用いるガイドライン（ACMG or ACGS）やアレル頻度データベースの選択も可能

Variant Info				Identified_variants...				Variant Info				Identified_variants...			
Chr:Pos	Ref/Alt	VAF	DP	Chr:Pos	Ref/Alt	VAF	DP	Gene Name	HGVS pDot	ACMGClassificatio...	Classification	Chr:Pos	Ref/Alt	VAF	DP
10:8116242	-/A	0.379473	1068	10:8116242	-/A	0.379473	1068	GATA3	?	PM2,BP4,BP7	Likely Benign	10:8116242	-/A	0.379473	1068
10:8116598	G/A	0.536101	554	10:8116598	G/A	0.536101	554	GATA3	?	BA1,BP4,BP7,BP6	Benign	10:8116598	G/A	0.536101	554
10:89720636	T/C	1	191	10:89720636	T/C	1	191	PTEN	?	PM2,BP4,BP7	Likely Benign	10:89720636	T/C	1	191
10:89720873	AAGGTCAGT...	0.507937	193	10:89720873	AAGGTCAGT...	0.507937	193	PTEN	NP_000305.3...	PM2,PVS1 Strong	Likely Pathogenic	10:89720873	AAGGTCAGT...	0.507937	193



評価結果のアノテーション

VarSeq

Inputs | Gene Thresholds | Consortium Sources | Frequency Sources | Control Sources

Classification System:  
 ACMG Variant Classification [Richards et al. 2015](#)  
 The ACMG criteria are tallied using a series of published rules into the resulting classification. A suggested update to these rules (Ellard et al.) changes how the Pathogenic and Likely Pathogenic classifications are reached.  
 Note the updated PVS1 rules are always used:  
 Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion [Tayoun et al. 2018](#)

ACMG

Algorithm Dependencies:  
 The ACMG classifier depends on the following sources and will add them automatically to the project as dependencies if they are not found:

- Conservation Scores Exonic, GHI Annotation **Already Exists**
- SIFT and PolyPhen2 Missense Predictions, GHI Annotation **Already Exists**
- ClinVar 2021-02-04, NCBI Annotation **Already Exists**
- Annotate Transcripts Algorithm with RefSeqGenes-NCBI **Already Exists**

Internal Database of Classified Germline ACMG Variants for Samples:  
 (Don't lookup previous classifications) Create ⓘ

## ■ グラフィカル画面でバリエントごとに評価を行う場合

- バリエントごとに評価を行う場合は、VCFファイル内の全バリエントを、評価に用いる少数のバリエントに絞り込みが必要
- VCFファイルに含まれていないバリエントの場合は、手動でバリエント情報を入力し、評価に用いることも可能

Add Variants for ACMG Sample 1

Variants to Select:

Filter Variants (Variants)

<input checked="" type="checkbox"/>	Variant	GT
<input checked="" type="checkbox"/>	PRDM16 p.H928P	● ○
<input checked="" type="checkbox"/>	POTEF p.V803I	● ○
<input checked="" type="checkbox"/>	RAF1 p.S257L	● ○
<input checked="" type="checkbox"/>	MLH1 p.Q701=	● ○
<input checked="" type="checkbox"/>	EGFR p.A763_Y764insFQEA	● ○
<input checked="" type="checkbox"/>	PTEN p.S385*	● ○
<input checked="" type="checkbox"/>	SALL4 p.E407D	● ○
<input checked="" type="checkbox"/>	CBS p.I278Tfs*16	● ○

Allow Reference Genotypes

Select All Clear All Prepare to Add

バリエントフィルタリング結果より選択

Add Variants for ACMG Sample 1

Enter Variant:

SMAD4 I500V

e.x. BRAF V600E [show more](#)

Variants Matching Query:

SMAD4 c.1498A>G 0.00% dbSNP ClinVar

SMAD4 c.1498\_1500d...

SMAD4 c.1498\_1500d...

SMAD4 c.1498\_1500d...

Sample Zygosity

Ref  Heterozygous  Homozygous

Allele Counts

Alt # alts Ref # refs VAF Percent %

Father: Mother:

Prepare to Add

バリエントを手動で入力

- スタート画面で、各バリエーションごとにサマリー情報と、該当する評価項目を確認
- 各評価項目より、評価に関する質問の画面にジャンプする

The screenshot displays the Variant Dashboard interface for a variant in the *RAF1* gene. The main content area is titled "Evidence Summary" and includes a "Variant Summary" section, a "Recommended to Score Pathogenic" section, and a "Variant Evidence" section. A red box highlights the "Recommended to Score Pathogenic" section, which contains six criteria (PM2, PM1, PP2, PP3, PS1, PM5) with checkboxes and arrows. A red arrow points from the text "評価項目" (Evaluation Items) to this section. The "Variant Evidence" section shows the variant's position on chromosome 3 (chr3) at 12,645,699, with a Phred Quality Score of 99.00 and a genotype of Heterozygous. The right sidebar shows the variant's details, including the mutation (G > A), dbSNP (rs80338796), ClinVar (13957), and ACMG Scoring criteria.

**Evidence Summary**

Variant Summary:  
The missense variant NM\_001354689.3(RAF1):c.770C>T (p.Ser257Leu) is not observed in the large population cohorts of gnomAD All, or 1kG All (Genome Aggregation Database et al., 2020:1000 Genomes). The variant was added to dbSNP as rs80338796 in version 131. This variant was found in ClinVar (Variant 13957) with a classification of Pathogenic and a review status of (3 stars) reviewed by expert panel. There is a large physicochemical difference between serine and leucine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties.

Recommended to Score Pathogenic

- PM2 → The p.Ser257Leu variant is novel (not in any individuals) in gnomAD All. The p.Ser257Leu variant is novel (not in any individuals) in 1kG All.
- PM1 → 25 variants within 6 amino acid positions of the variant p.Ser257Leu have been shown to be pathogenic, while none have been shown to be benign.
- PP2 → The gene *RAF1* has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 2.46. The gene *RAF1* contains 40 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene.
- PP3 → The p.Ser257Leu missense variant is predicted to be damaging by both SIFT and PolyPhen2. The serine residue at codon 257 of *RAF1* is conserved in all mammalian species. The nucleotide c.770 in *RAF1* is predicted conserved by GERP++ and PhyloP across 100 vertebrates.
- PS1 → The p.Ser257Leu variant is a missense mutation resulting in an amino acid change which is shared by the previously classified pathogenic variant p.S257L.
- PM5 → The p.Ser257Leu variant is a missense mutation resulting in an amino acid change which occurs at the same amino acid position as 2 previously classified pathogenic variants.

**Variant Evidence**

Variant Evidence for ACMG Sample 1

Chromosome: chr3 Position: 12,645,699

NC\_000003.11 (GRCh37 Chr3): g.12645699G>A

Allele	DP	%
G	0	
A		

Genotype: Heterozygous

Phred Quality Score: 99.00  
1 in 1,000,000 probability of FP

**Variant** Sections 2

GRCh37: chr3: 12,645,699 Mutation: G > A

Genotype: Heterozygous Allele Ratio:

dbSNP: rs80338796 ClinVar: 13957

Gene: *RAF1* Transcript: NM\_001354689.3...

NM\_001354689.3: c.770C>T NP\_001341618.1: p.S257L

Effect: Missense (missense\_variant) Exon: 7 of 18

**ACMG Scoring**

Scored Criteria:  
Probability of Pathogenic:

- 各評価項目の質問に回答すると、最終的な評価スコアが計算される
- 各評価項目では、VSClinicalが判断した適切な回答へのマークや、判断の根拠などが確認できる

<b>BS1</b> Allele frequency is greater than expected for disorder <input type="radio"/> Yes <input type="radio"/> No <input checked="" type="radio"/> Unanswered	<b>Caveats:</b> <input type="checkbox"/> Poor coverage region in target capture <input type="checkbox"/> Insertions/deletions may not be called by consortium pipeline
<b>Reasons for No:</b> <ul style="list-style-type: none"><li>• The variant p.Ile500Val occurs in 1 individual in gnomAD</li><li>• The variant p.Ile500Val occurs in 0 individuals in 1kG</li></ul>	<b>Evidence:</b> <b>Strong</b> Supporting <a href="#">See further discussions on BS1</a> <b>Comments:</b> <input type="text"/>

<b>PM2</b> Absent from controls in population catalogs Or if recessive, at extremely low frequency. Example population catalogs include 1000 Genomes and gnomAD. <input type="radio"/> Yes <input type="radio"/> No <input checked="" type="radio"/> Unanswered	<b>Caveats:</b> <input type="checkbox"/> Poor coverage region in target capture <input type="checkbox"/> Insertions/deletions may not be called by consortium pipeline
<b>Reasons for Yes:</b> <ul style="list-style-type: none"><li>• The p.Ile500Val variant is observed in 1/113,754 (0.0009%) alleles from individuals of gnomAD Non Finnish European background in gnomAD.</li><li>• The p.Ile500Val variant is novel (not in any individuals) in 1kG.</li></ul>	<b>Evidence:</b> <b>Moderate</b> Supporting A supporting strength may be chosen if variant follows disorder prevalence <a href="#">See further discussions on PM2</a> <b>Comments:</b> <input type="text"/>

- すべての評価項目に回答すると、クリアした評価項目に基づき、臨床的解釈のテキストが自動生成される
- 必要に応じて、Primary Findings / Secondary Findingsの区別や、疾患名などとともにレポート出力が可能
- レポートには、NGS実験の各種QCデータや、評価に用いたデータベース名、参考論文などの情報を追加が可能
  - ✓ Coverage Summary
  - ✓ Variant Summary
  - ✓ Gene Coverage
  - ✓ Annotation Sources
  - ✓ Inline References ...など

### Variant Interpretation for Sample ACMG Sample 1

Exclude Variant:  Dismiss/Fail Variant 🗑️ Delete...

Reporting As:

Variant Sets:  
 Primary Findings (Variants)  
 Secondary Findings (Variants)  
 Uncertain Significance (Variants)

Classification:

For Disorder:   
OMIM: [OMIM ID](#) 🔗 MONDO: [Mondo ID](#) 🔗

Inheritance/Variant Type:

Interpretation:

The missense variant NM\_001354689.3(RAF1):c.770C>T (p.Ser257Leu) causes the same amino acid change as a previously established pathogenic variant. The p.Ser257Leu variant is novel (not in any individuals) in gnomAD All. The p.Ser257Leu variant is novel (not in any individuals) in 1kG All. There is a large physicochemical difference between serine and leucine, which is likely to impact secondary protein structure as these residues differ in polarity, charge, size and/or other properties. The gene RAF1 has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 2.46. The gene RAF1 contains 40 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. 25 variants within 6 amino acid positions of the variant p.Ser257Leu have been shown to be pathogenic, while none have been shown to be benign. The p.Ser257Leu missense variant is predicted to be damaging by both SIFT and PolyPhen2. The serine residue at codon 257 of RAF1 is conserved in all mammalian species. The nucleotide c.770 in RAF1 is predicted conserved by GERP++ and PhyloP across 100 vertebrates. For these reasons, this variant has been classified as Pathogenic.

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