

# 病的バリエーションの分類・解釈の自動化

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- 次世代シーケンサーで検出されたバリエーションのリストより、病的なバリエーションを抽出するには、文献やデータベース検索を駆使しながら、バリエーションごとの病原性を評価する必要があり、非常に手間がかかります。
- Golden Helix社VarSeq<sup>®</sup>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です。

The screenshot displays the ACMG Classification interface for the variant RAF1 c.770C>T (p.S257L). The classification is **PATHOGENIC**. The scored criteria include PS1, PM1, PM2, PM5, PP2, and PP3. Supporting evidence includes ClinVar: 3-star Pathogenic, Population: Absent from gnomAD, Hotspot: 18 pathogenic neighbors, and In-silico: All algorithms agree damaging. The pathogenicity score is 86.3% and 6 out of 28 criteria are met.

Pathogenicity	Criteria Met
86.3%	6 / 28

Annotation

Variant Filtering

The screenshot displays the VarSeq software interface. At the top, there's a 'GenomeBrowse' window showing a genomic track for chromosome 16, with coordinates 16: 2,495,470 to 16: 2,495,530. Below this, there are tracks for 'Reference Sequence GRCH37 g1k, 1000Genomes', 'RefSeq Genes 105, NCBI' (showing the CCNF gene), and 'OMIM Genes 2010-10-27, UCSC' (showing gene 600227). A 'Variants - NA12878' track shows several variants, including T/G and C/A. On the left, a 'Trio Analysis' panel shows various metrics like 'Compound Het', 'Proband Genotype Quality', and 'Proband Read Depth'. At the bottom, a 'Detail' window shows a table of variant sites.

Variant Sites	Genotypes	Classification	Compound Het Variants				
Chr:Pos	Ref/Alt	Identifier	Proband (NA12878)	Mother (NA12891)	Father (NA12892)	Sequence Ontology	Is CH? Inherited From
11:108183167	A/G	rs659243	G,G	G,G	G,G	missense_variant	False NA
13:49033835	G/A	rs20211...	A,G	A,G	A,G	missense_variant	False NA
14:24567498	A/C	rs30211...	C,C	C,C	C,C	missense_variant	False NA
14:73664751	T/G	rs19972...	G,T	G,T	G,T	missense_variant	False NA
14:106208082	G/T	rs11621...	G,T	T,T	T,T	missense_variant	False NA
16:2495482	T/G	rs20154...	G,T	T,T	G,T	missense_variant	True Father

Data Analysis

Genome Browser

■ 様々なデータベースを用いて、バリエーションデータ（VCFファイル）へアノテーション付けを実行

- RefSeq Genes
- dbSNP
- ClinVar
- OMIM
- CIViC
- ICGC / TCGA
- MSK Impact
- Orphanet
- BRCA Exchange
- PMKB
- dbNSFP
- REVEL
- CADD
- 1000 Genomes
- NHLBI 6500 Exomes
- ExAC Variant
- gnomAD Exomes/Genomes
- GenomeAsia 100K
- 各種遺伝子パネルのターゲットデータ ...など

■ アノテーション付けされたバリエーションデータより、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

■ ゲノムブラウザーにより、サンプルのバリエーションデータやリードアライメントデータ（BAM/CRAMファイル）、また各種アノテーションを可視化

■ 有償アドオンによる機能拡張で、CNV検出や臨床的意義の自動評価、パイプライン機能などが利用可能



## 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、病源性 (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、臨床エビデンス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臨床的意義の評価に使用
- 各種バイオマーカー (SNV, InDels, CNV, 融合遺伝子, TMB/MSIなど) をAMPガイドラインのエビデンスレベルで分類し、がんの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
- 体細胞バリエントの腫瘍原性 (Oncogenicity) をスコアで評価

## ■ 病原性の評価方法

- 一般集団内のアレル頻度
- 既知の臨床エビデンス
- 遺伝子機能への影響
- バイオインフォマティクス解析による機能予測
- 家族歴

## ■ 評価項目と結果

- 33種類の評価項目
- 評価スコアの合計より、バリアントの病原性を5段階に分類
  - Pathogenic
  - Likely Pathogenic
  - VUS (Variant of Uncertain Significance)
  - Likely Benign
  - Benign

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
<b>Population Data</b>	MAF is too high for disorder <i>BS1/BS1</i> OR observation in controls <i>INCONSISTENT WITH</i> disease penetrance <i>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>PVS1</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>				<i>De novo</i> (without paternity & maternity confirmed) <i>PM6</i>	<i>De novo</i> (paternity & maternity confirmed) <i>PS2</i>	
<b>Allelic Data</b>		Observed in <i>trans</i> with a dominant variant <i>BP2</i> Observed in <i>cis</i> with a pathogenic variant <i>BP2</i>		For recessive disorders, detected in <i>trans</i> 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>			

#CHROM	POS	ID	REF	ALT
1	569492	rs6594033	T	C
4	4927684	rs1264288	A	G
10	4586828	rs1465531	G	A

⋮



Variant 1	Benign
Variant 2	Pathogenic
Variant 3	Likely Pathogenic

⋮

バリエントリスト

評価結果

- VS Clinicalでは、バリエントのリストを読み込むだけで、自動でバリエントの評価を実行
- バリエントリストはVCFファイルまたは、バリエント名の手動入力に対応

## Evidence Summary

### Variant Summary:

The missense variant NM\_001354689.3(RAF1):c.770C>T(p.S257L) is not observed in the large population cohorts of gnomAD, or 1kG (Genome Aggregation Database et al., 2020;1000 Genomes Consortium et al., 2015). 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. The p.Ser257Leu variant is novel (not in any individuals) in 1kG.
- ✓ PM1 → 30 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 2.49. The gene RAF1 contains 56 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 3 previously classified pathogenic variants.

検出された評価項目

- VSClinicalでバリエントの評価を行うと、バリエントごとに評価項目が自動で検出される
- 評価項目のサマリーテキストでは、アノテーションデータベースの照合結果などをまとめたエビデンス情報なども確認が可能

## ■ 評価結果の出カデータ

- VSClinicalでは、パスした評価項目のスコア、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

評価項目の  
集計データ

ACMG Classification:

**Pathogenic**

Rule Pathogenic (iii): 1 Strong AND ≥3 Moderate, OR 2 Moderate AND ≥2 Supporting, OR 1 Moderate AND ≥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

バリアントの病原性  
評価の分類

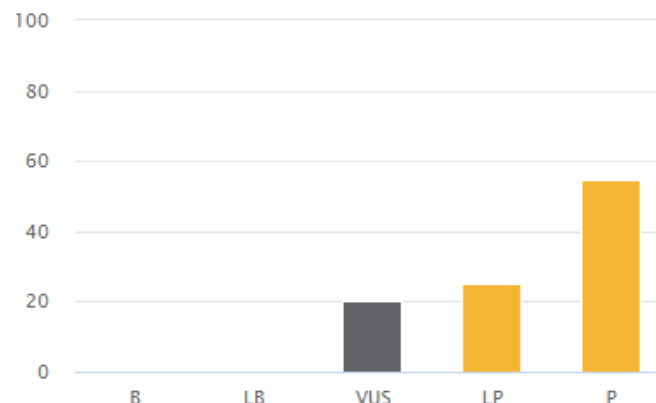
## Golden Helix Classification Prediction

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.

## ■ 遺伝子データベース

- RefSeq

## ■ アレル頻度データベース

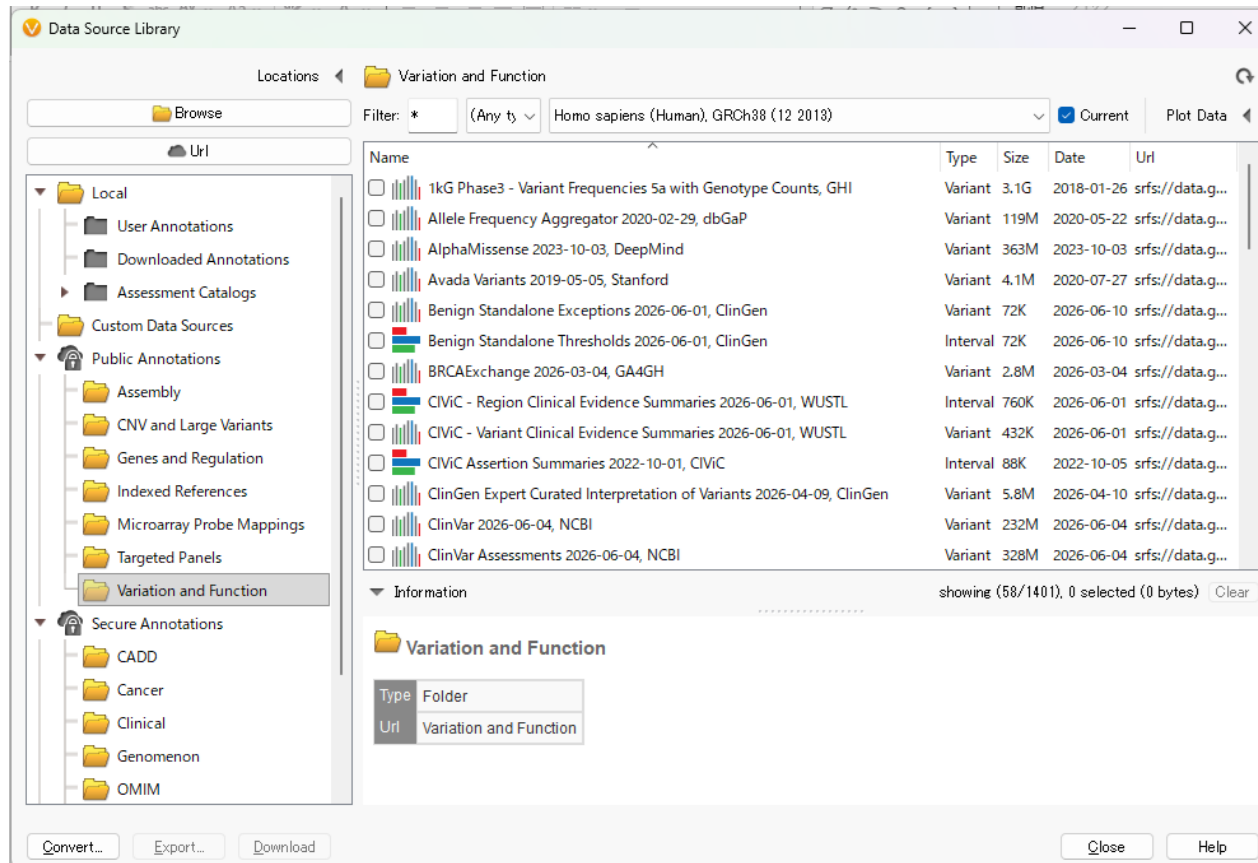
- 1000Genome
- gnomAD

## ■ 疾患ゲノムデータベース

- ClinVar
- ClinGen
- OMIM
- Orphanet

## ■ 機能予測データベース

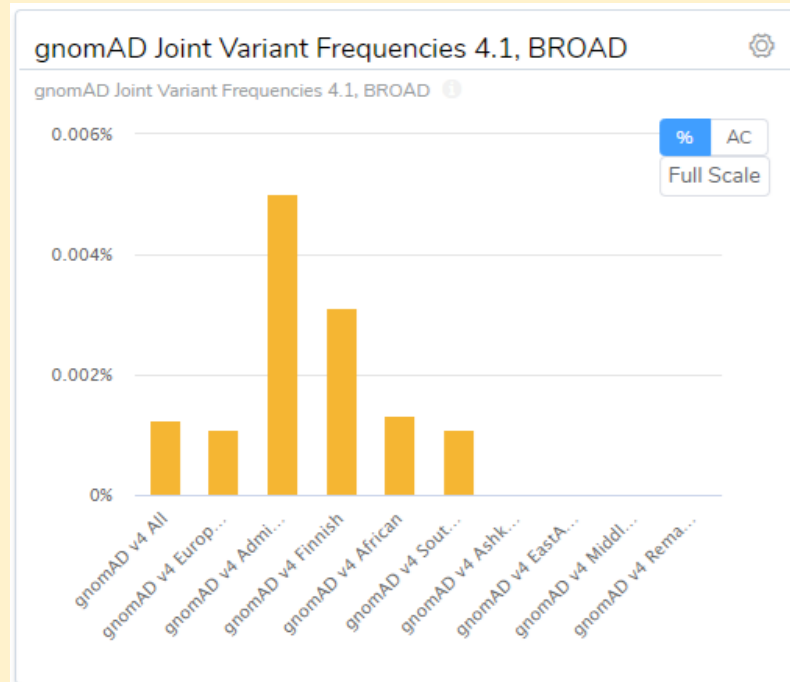
- SIFT
- Polyphen2
- Conservation Score
- CADD
- GeneSplicer ...など



- 評価を行うのに必要なアノテーションデータベースは、最新のものが自動的にダウンロードされ使用される

- ソフトウェアのアノテーションライブラリーより、任意のアノテーションデータベースをダウンロードし、アノテーション付けに使用することも可能

## 一般集団内のアレル頻度による評価



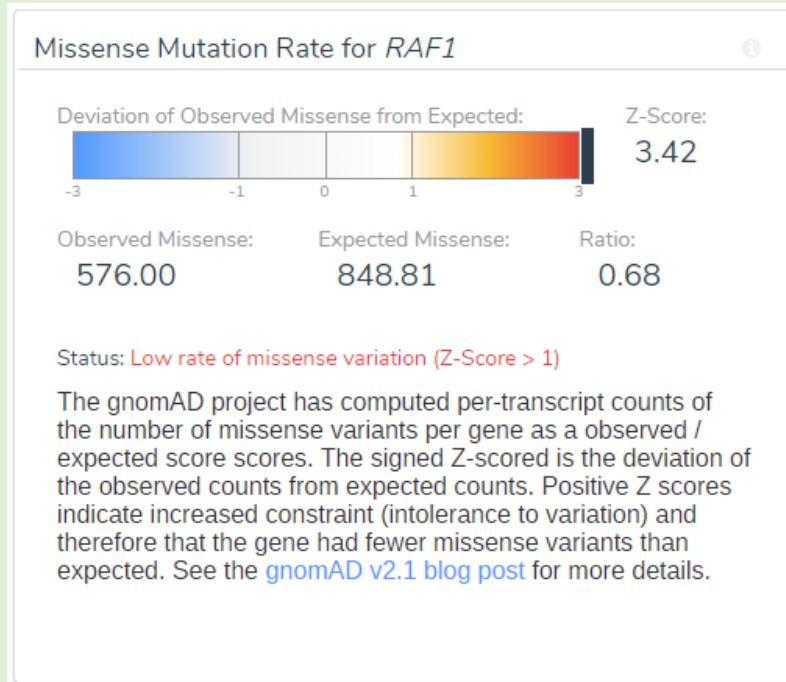
- gnomAD, 1000 Genomesのアレル頻度データベースをもとに、バリアントの一般集団内のアレル頻度を確認
- 集団内のアレル頻度が高いバリアントは良性、データベースに存在しないか、または頻度の低いものは病的と評価する

## 遺伝子上の位置情報を用いた評価

Variant Type:		Within Same:		Classification:		Rating:
All	Missense	Gene	Exon	All	Benign	Pathogenic
	LoF		Codon			
Dist AA/bp	Variant	Exon	Effect	Sources	Clinical Significance	Rating/Last Seen
-1 / -2	c.768G>C (p.R256S)	7 / 17	Missense	ClinVar	Pathogenic	★ ★ ★ ★
-1 / -2	c.768G>T (p.R256S)	7 / 17	Missense	ClinVar	Likely pathogenic	★ ★ ★ ★
0 / -1	c.769T>A (p.S257T)	7 / 17	Missense	ClinVar	Likely pathogenic	★ ★ ★ ★
0 / -1	c.769T>C (p.S257P)	7 / 17	Missense	ClinVar	Likely pathogenic	★ ★ ★ ★
0 / 0	c.770C>G (p.S257W)	7 / 17	Missense	ClinVar	Uncertain Significance	★ ★ ★ ★
0 / 0	c.770C>T (p.S257L)	7 / 17	Missense	ClinVar	Pathogenic	★ ★ ★ ★
0 / 1	c.771G>A (p.S257=)	7 / 17	Synonymous	ClinVar	Likely benign	★ ★ ★ ★
1 / 2	c.772A>T (p.T258S)	7 / 17	Missense	ClinVar	Uncertain Significance	★ ★ ★ ★
1 / 3	c.773C>G (p.T258R)	7 / 17	Missense	ClinVar	Likely pathogenic	★ ★ ★ ★
1 / 4	c.774A>G (p.T258=)	7 / 17	Synonymous	ClinVar	Likely benign	★ ★ ★ ★

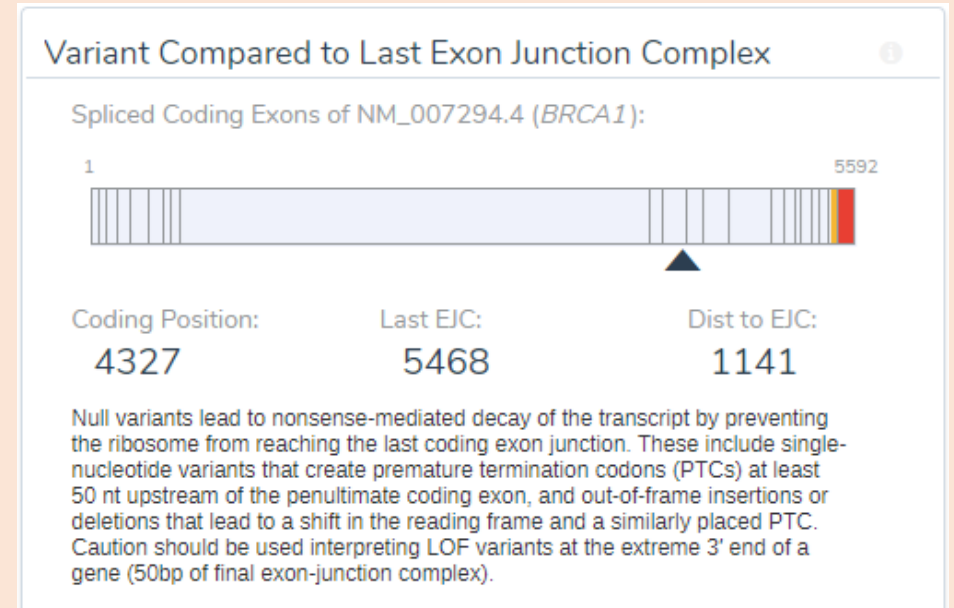
- バリアントが、遺伝子のホットスポットや機能ドメイン上に存在するかどうかや、近傍に良性バリアントが存在するかどうかを確認
- ホットスポット上に存在する場合や、同じコドン領域に良性のバリアントが他に存在しない場合、病的と評価する

## ミスセンスバリアントの評価



- データベース登録されている良性のミスセンスバリアントの割合や、コンピュータ予測によって、ミスセンスバリアント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アントが多く、なおかつタンパク質の短縮を引き起こすバリアントが疾患の原因だと考えられる場合は、ミスセンスバリアントは良性と判定される

## ナンセンスバリアントの評価



- 同じ遺伝子上で、評価対象のバリアントより下流に、病原性のナンセンスバリアント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、バリアント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、良性とする
- 遺伝子ごとに、ナンセンスバリアントの実測値／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

## コンピュータ予測による評価

MSA-SIFT	Damaging		1.00 (greater than 0.95)
MSA-PolyPhen2	Damaging		1.000 (greater than 0.446)
PhyloP	Conserved		9.81 (greater than 2)
GERP++	Conserved		19.50 (greater than 10)
Combined Annotation Dependent Depletion (CADD) Score: ⓘ			
CADD	Uncertain		3.22 (between 2 and 5)
Disrupting Nearby Splice Predictions: ⓘ			
GeneSplicer	Disrupted		0.59 (delta -0.40)
MaxEntScan	Disrupted		0.01 (delta -0.94)
NNSplice	Disrupted		0.32 (delta -0.60)
PWM	Disrupted		0.59 (delta -0.12)

- 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、バリアントの有害性を評価する
- スプライシング異常を引き起こすバリアントについては、GeneSplicerなどのスプライス部位予測用ツールが使用される

## 既知の臨床エビデンスによる評価

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)

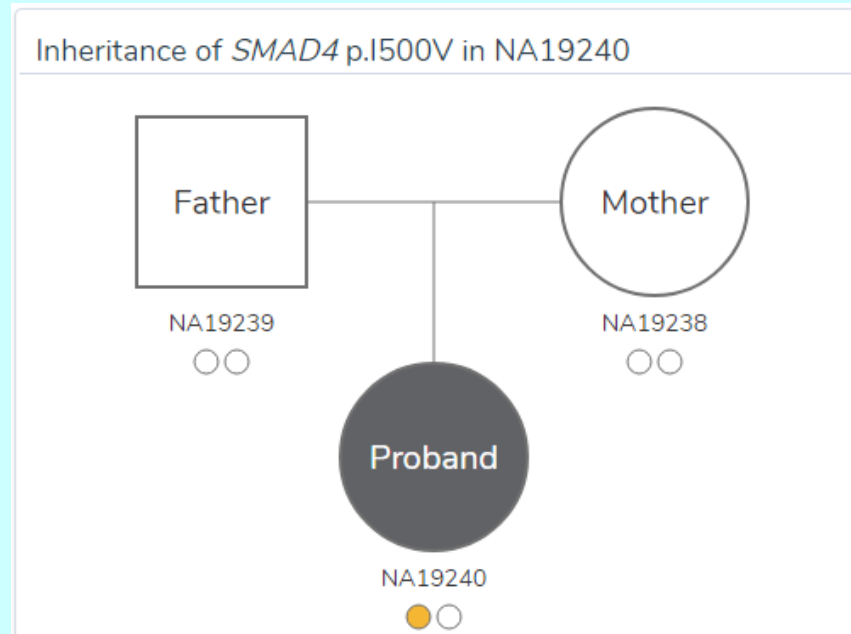
Disease: Noonan Syndrome 5; Leopard Syndrome 2; Noonan Syndrome with Multiple Lentiginos; Rasopathy; Noonan Syndrome; Not Provided; Lung Adenocarcinoma; Adenocarcinoma of Stomach; Malignant Melanoma of Skin; Neoplasm of the Large Intestine; Cardiomyopathy, Dilated, 1nn;leopard Syndrome 2;noonan Syndrome 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. ⓘ

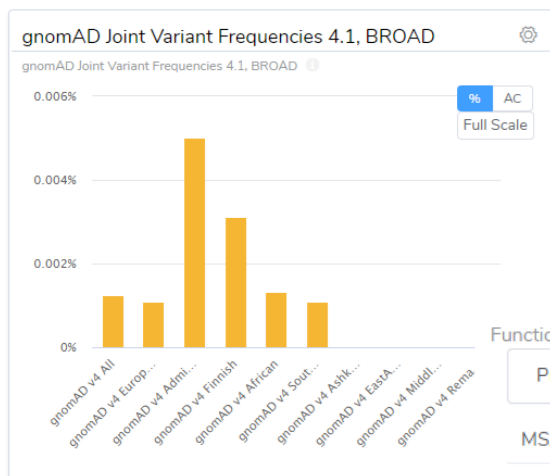
- データベースで病的バリアントと登録されているものと同じアミノ酸置換を引き起こすバリアントや、遺伝子上の同じ位置に存在し、異なるアミノ酸置換を引き起こすバリアントにを評価する
- データベース登録されているレコード情報や、出典論文情報なども確認が可能

## 家族歴を利用した評価



- 両親には存在せず、罹患者のみに存在するバリエント（de Novo バリエント）の場合は、病的と評価される
- 両親がバリエント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評価となる

評価対象のバリエントを入力



Functional Predictions:

Primates Mammals **Vertebrates**

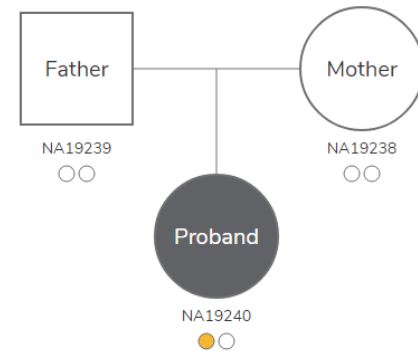
MSA-SIFT	Damaging		1.00 (greater than 0.95)
MSA-PolyPhen2	Damaging		1.000 (greater than 0.446)
PhyloP	Conserved		9.81 (greater than 2)
GERP++	Conserved		19.50 (greater than 10)
Combined Annotation Dependent Depletion (CADD) Score:			
CADD	Uncertain		3.22 (between 2 and 5)

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; Multiple Lentiginos; Noonan Syndrome 1  
Source: Invitae

Inheritance of *SMAD4* p.I500V in NA19240



各種データベースと自動的に照合



評価スコアを算出

## ■ 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
- 詳細データの確認や評価スコアの調整などは不可

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

## ■ VS Clinical Variant Dashboard

- 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を行う
- 評価に用いるデータベースや文献などで根拠を確認しながらの評価が可能
- 評価スコアの調整なども可能

### Evidence Summary

#### Variant Summary:

The missense variant NM\_001354689.3(RAF1):c.770C>T(p.S257L) is not observed in the large population cohorts of gnomAD All, or 1kG All (Genome Aggregation Database et al., 2020; 1000 Genomes Consortium et al., 2015). 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** 30 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 2.49. The gene RAF1 contains 56 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 3 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

## ■ ACMG Sample Classifierから実行する場合

- ACMG Sample Classifierを実行すると、バリエントリストにACMG評価結果の情報がアノテーションとして付加される
- 全バリエントより、任意の評価結果（例：Pathogenicのみ）のバリエントのフィルタリングが可能

Variant Info		HD200-rep1			
Chr:Pos	Ref/Alt	Variant Allele Fraction	Read Depths (DP)	Genotype Qualities (GQ)	Zygosity
17:36097775	G/A	0.780488	41	35	Heterozygous
17:36098040	G/A	0.589091	275	574	Heterozygous
17:36101156	T/C	0.560897	312	747	Heterozygous
17:41234451	G/A	0.241218	427	?	Heterozygous
17:47436514	G/A	0.287129	101	81	Heterozygous
17:56773834	T/C	0.282609	92	69	Heterozygous
17:59761706	A/G	0.666667	84	125	Heterozygous
17:59820068	G/A	0.684211	57	91	Heterozygous
17:59820170	C/A	0.69281	153	131	Heterozygous
17:59857275	C/A	0.725	80	62	Heterozygous



ACMG Sample Classifierによるアノテーション付け

Variant Info		HD200-rep1				ACMG Sample Classifier for HD200-rep1				
Chr:Pos	Ref/Alt	Variant Allele Fraction	Read Depths (DP)	Genotype Qualities (GQ)	Zygosity	Gene Name	Gene Inheritance	Transcript Name	ACMG Classification Criteria	Classification
17:36097775	G/A	0.780488	41	35	Heterozygous	HNF1B	Dominant	NM_000458.4	BA1,BS2,BP7	Benign
17:36098040	G/A	0.589091	275	574	Heterozygous	HNF1B	Dominant	NM_000458.4	BA1,BS2,BP7,BP6	Benign
17:36101156	T/C	0.560897	312	747	Heterozygous	HNF1B	Dominant	NM_000458.4	BA1,BS2,BP7	Benign
17:41234451	G/A	0.241218	427	?	Heterozygous	BRCA1	Dominant	NM_007294.4	PM2,PVS1,PP5	Pathogenic
17:47436514	G/A	0.287129	101	81	Heterozygous	ZNF652	Default (Recessive)	NM_001145365.3	BA1,BS2,BP7	Benign
17:56773834	T/C	0.282609	92	69	Heterozygous	RAD51C	Recessive	NM_058216.3	BA1,BS2,BP7,BP6	Benign
17:59761706	A/G	0.666667	84	125	Heterozygous	BRIP1	Dominant	NM_032043.3	BA1,BS2,BP7,BP6	Benign
17:59820068	G/A	0.684211	57	91	Heterozygous	BRIP1	Dominant	NM_032043.3	BA1,BS2,BP7,BP6	Benign
17:59820170	C/A	0.69281	153	131	Heterozygous	BRIP1	Dominant	NM_032043.3	BA1,BS2,BP7,BP6	Benign
17:59857275	C/A	0.725	80	62	Heterozygous	BRIP1	Dominant	NM_032043.3	BA1,BS2,BP7	Benign

Add Variants for ACMG Sample 1

Sample: < 1 of 1 > ACMG Sample 1 (Cu

Variants to Select:

Filter Variants (Variants)

<input checked="" type="checkbox"/>	Variant	GT
<input checked="" type="checkbox"/>	RAF1 p.S257L	○
<input type="checkbox"/>	EGFR p.A763_Y764insFQEA	○
<input type="checkbox"/>	PTEN p.S385*	○
<input type="checkbox"/>	CBS p.I278Tfs*16	○

Allow Reference Genotypes

Select All Clear All Prepare to Add

OR

Add Variants for ACMG Sample 1

Sample: < 1 of 1 > ACMG Sample 1 (Cu

Enter Variant:

RAF1 S257L

e.x. BRAF V600E show more

Variants Matching Query:

RAF1 c.770C>T dbSNP ClinVar

RAF1 c.770\_771delins...

RAF1 c.769\_770delins...

RAF1 c.769\_771delins...

RAF1 c.769\_771delins...

RAF1 c.769\_771delins...

Sample Zygosity

Ref  Heterozygous  Homozygous

Read Depths

Alt # alt Total # total VAF Percent %

Father: Mother:

Prepare to Add

バリエントテーブルから選択

バリエント名を手動で入力

Recommended to Score Pathogenic

- PM2 → The p.Ser257Leu variant is novel (not in any individuals) in gnomAD v4 All. The p.Ser257Leu variant is novel (not in any individuals) in 1kG All.
- PM1 → 32 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 3.42. The gene RAF1 contains 59 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 3 previously classified pathogenic variants.

バリエントに該当する評価項目と結果を自動検出

## ■ VSClinical Variant Dashboardから実行する場合

- 評価に用いるバリエントは、VCFファイルよりインポートしたバリエントテーブルより選択するか、キーボードでバリエント名を直接入力することも可能
- バリエントを指定すると、該当する評価項目が自動で検出され、評価結果のテキストとともに表示される

### Computational Evidence

Functional Predictions: Primates Mammals Vertebrates

MSA-SIFT	Damaging		1.00 (greater than 0.95)
MSA-PolyPhen2	Damaging		1.000 (greater than 0.446)
PhyloP	Conserved		9.81 (greater than 2)
GERP++	Conserved		19.50 (greater than 10)

Combined Annotation Dependent Depletion (CADD) Score: Uncertain

CADD	Uncertain		4.98 (between 2 and 5)
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Nearest Splice Site: **Donor** Distance to Splice Site: **65bp upstream**  
 Exon 7 of 17 Exonic

Variant is too far from a splice site to have predictions

[Open Splice Site Analysis on Region](#)

### 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
Squid CC CTC TCC CAG AGG CAG AGA TCG ACA TCC ACA CCT AAT GTC C
```

← データベースの照合結果

**PP3** **BP4**

Multiple lines of computational evidence support a deleterious effect on the gene or gene product

Examples include in-silico protein function predictions, conservation, splicing impact, etc.

All deleterious

All no impact

Uncertain

Caveats:

- Computation evidence evaluating transcript used in interpretation
- Complete agreement can be expected or disagreement justified
- Gene has recently evolved in humans (expected to be non-conserved)

PP3 scored by Takuya Ozawa a few seconds ago

Evidence: Strong Moderate Supporting

Comments:

Reasons for All Deleterious:

- 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.

Reasons for All No Impact:

- The p.Ser257Leu variant is not predicted to introduce a novel splice site by any splice site algorithm.

評価結果

評価の根拠

- 各評価項目をクリックすると、データベースとの照合結果と評価結果を確認可能
- 評価を下した根拠などの情報も、同時に表示される
- 評価結果は手動で編集することも可能

Scoring System:  
**ACMG Variant C**  
 To change the classific

Scored Criteria by Streng

**Pathogenic**

**Benign**

ACMG Classification:  
**Pathogenic**  
 Rule Pathogenic (i): 1 Very strong Supporting

### Golden Helix Classification Prediction

Probability of Pathogenic given Scored Criteria:

Status: Probability of 89.2% predicting classification of **Pathogenic**

Probability for Each Classification:

Classification	Probability (%)
B	0
LB	0
VUS	20
LP	25
P	55

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 PVS1 PP5**  
 Previously Saved: PM2 PVS1 PP5

Evidence for Pathogenic: [Interpretation](#) [Evidence](#) [Comments](#)

The stop gained NM\_000425.5(L1CAM):c.2380C>T (p.Gln794Ter) has been reported to ClinVar as Pathogenic/Likely pathogenic with a status of (2 stars) criteria provided, multiple submitters, no conflicts (Variation ID 226120 as of 2024-05-03). The p.Gln794Ter variant is novel (not in any individuals) in 1kG All. The p.Gln794Ter variant is novel (not in any individuals) in gnomAD v4 All. This variant is predicted to cause loss of normal protein function through protein truncation. This variant is a stop gained variant which occurs in an exon of L1CAM upstream of where nonsense mediated decay is predicted to occur. This variant has been previously classified as pathogenic, indicating that the region is critical to protein function. There are 46 downstream pathogenic loss of function variants, with the furthest variant being 419 residues downstream of this variant. This indicates that the region is critical to protein function. The gene L1CAM has a low rate of benign loss of function variants as indicated by a low upper bound of the observed/expected confidence interval 0.13. The p.Gln794Ter variant is a loss of function variant in the gene L1CAM, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP\_000416.1:p.V8Gfs\*24 and 71 others. For these reasons, this variant has been classified as Pathogenic. ⓘ

[Add to Interpretation](#)

## レポート用テキスト

## 評価結果サマリー

- 評価結果の編集の完了後、各項目が病原性の可能性を示唆するスコアや良性の可能性を示唆するスコア、最終的な判定結果をまとめたサマリーを表示
- 同時にレポートに記載される臨床的解釈のテキストも出力

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