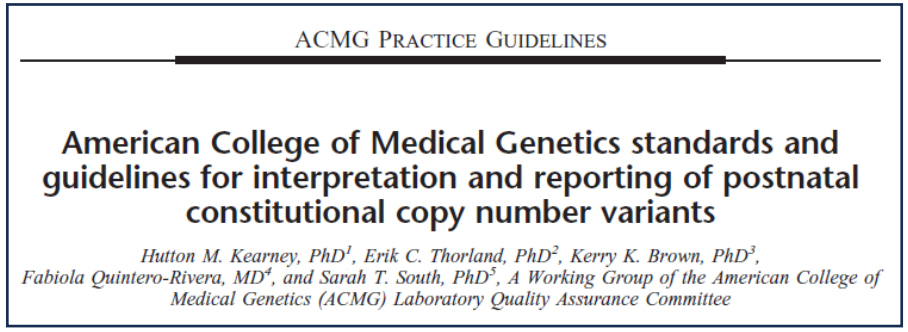
マイクロアレイ染色体検査普及のための産学連携コンソーシアム主催
マイクロアレイ染色体検査解釈ハンズオンウェビナー 2023.9.19

マイクロアレイ染色体検査解釈
ハンズオンウェビナー

中級編　解説資料
解釈概要

静岡県立こども病院　清水健司

CNV解釈体系の歴史と主要論文


ACMG Practice Guidelines

American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants

Hutton M. Kearney, PhD[1], Erik C. Thorland, PhD[2], Kerry K. Brown, PhD[3], Fabiola Quintero-Rivera, MD[4], and Sarah T. South, PhD[5], A Working Group of the American College of Medical Genetics (ACMG) Laboratory Quality Assurance Committee

2011　初の体系的解釈　３段階病原性分類

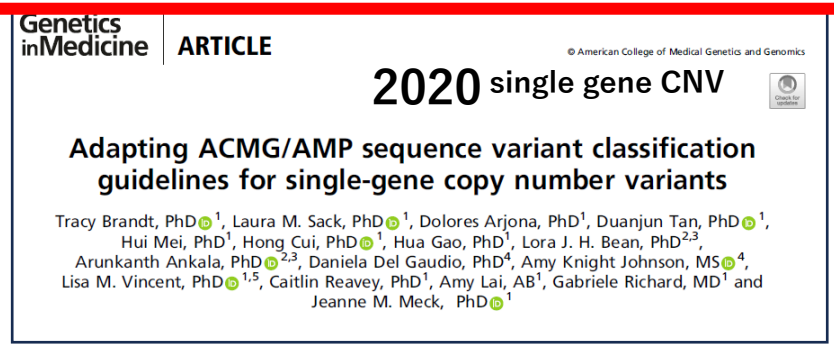
UPDATE


© American College of Medical Genetics and Genomics　ACMG TECHNICAL STANDARDS　Genetics inMedicine

2019 5段階評価＋網羅的解釈

Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen)

Erin Rooney Riggs, MS, CGC[1], Erica F. Andersen, PhD[2,3], Athena M. Cherry, PhD[4], Sibel Kantarci, PhD[5], Hutton Kearney, PhD[6], Ankita Patel, PhD[7], Gordana Raca, MD, PhD[8], Deborah I. Ritter, PhD[9], Sarah T. South, PhD[10], Erik C. Thorland, PhD[6], Daniel Pineda-Alvarez, MD[11], Swaroop Aradhya, PhD[4,11] and Christa Lese Martin, PhD[1]


Genetics inMedicine　ARTICLE　© American College of Medical Genetics and Genomics

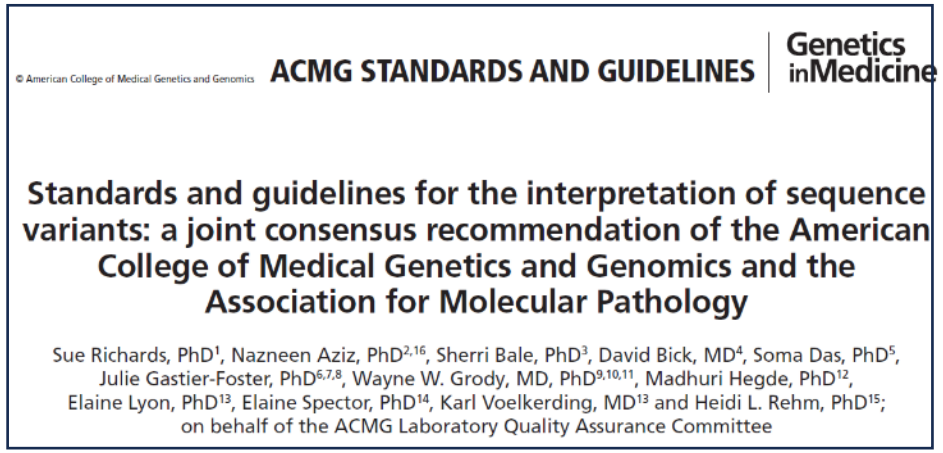
2020 single gene CNV

Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants

Tracy Brandt, PhD[1], Laura M. Sack, PhD[1], Dolores Arjona, PhD[1], Duanjun Tan, PhD[1], Hui Mei, PhD[1], Hong Cui, PhD[1], Hua Gao, PhD[1], Lora J. H. Bean, PhD[2,3], Arunkanth Ankala, PhD[2,3], Daniela Del Gaudio, PhD[4], Amy Knight Johnson, MS[4], Lisa M. Vincent, PhD[1,5], Caitlin Reavey, PhD[1], Amy Lai, AB[1], Gabriele Richard, MD[1] and Jeanne M. Meck, PhD[1]

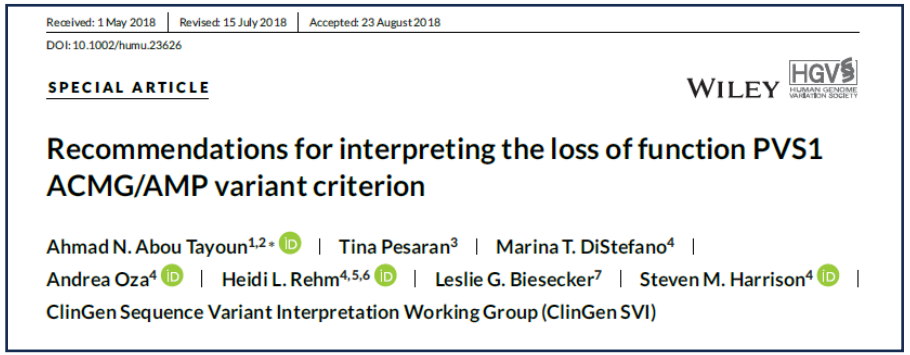
CNV解釈との融合

2015 網羅的解析進展に伴う
sequence variant guideline体系化


© American College of Medical Genetics and Genomics　ACMG STANDARDS AND GUIDELINES　Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee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DOI: 10.1002/humu.23626

SPECIAL ARTICLE　WILEY HGVS

Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion

Ahmad N. Abou Tayoun[1,2]* | Tina Pesaran[3] | Marina T. DiStefano[4] | Andrea Oza[4] | Heidi L. Rehm[4,5,6] | Leslie G. Biesecker[7] | Steven M. Harrison[4] | ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI)

2018
ハプロ不全遺伝子における
機能喪失疑いバリアントの体系的分類
（PVS1-criteria）

Table 1 CNV interpretation scoring metric: copy-number loss

Evidence type	Evidence	Suggested points/case	Max score
Section 1: Initial assessment of genomic content			
Copy-number loss content	**1A**. Contains protein-coding or other known functionally important elements.	0 (Continue evaluation)	0
	1B. Does NOT contain protein-coding or any known functionally important elements.	−0.60	−0.60
Section 2: Overlap with established/predicted haploinsufficiency (HI) or established benign genes/genomic regions ***(Skip to section 3 if your copy-number loss DOES NOT overlap these types of genes/regions)***			
Overlap with ESTABLISHED HI genes or genomic regions and consideration of reason for referral	**2A**. Complete overlap of an established HI gene/genomic region.	1.00	1.00
	2B. Partial overlap of an established HI genomic region • The observed CNV does NOT contain the known causative gene or critical region for this established HI genomic region OR • Unclear if known causative gene or critical region is affected OR • No specific causative gene or critical region has been established for this HI genomic region	0 (Continue evaluation)	0
	2C. Partial overlap with the 5′ end of an established HI gene (3′ end of the gene not involved)…	See categories below	
	2C-1. …and coding sequence is involved	0.90 *(range: 0.45 to 1.00)*	1.00
	2C-2. …and only the 5′ UTR is involved	0 *(range: 0 to 0.45)*	0.45
	2D. Partial overlap with the 3′ end of an established HI gene (5′ end of the gene not involved)…	See categories below	
	2D-1. …and only the 3′ untranslated region is involved.	0 (Continue evaluation)	0
	2D-2. …and only the last exon is involved. Other established pathogenic variants have been reported in this exon.	0.90 *(range: 0.45 to 0.90)*	0.90
	2D-3. …and only the last exon is involved. No other established pathogenic variants have been reported in this exon.	0.30 *(range: 0 to 0.45)*	0.45
	2D-4. …and it includes other exons in addition to the last exon. Nonsense-mediated decay is expected to occur.	0.90 *(range: 0.45 to 1.00)*	1.00
	2E. Both breakpoints are within the same gene (intragenic CNV; gene-level sequence variant).	See ClinGen SVI working group PVS1 specifications • PVS1 = 0.90 *(Range: 0.45 to 0.90)* • PVS1_Strong = 0.45 *(Range: 0.30 to 0.90)* • PVS1_Moderate or PM4 (in-frame indels) = 0.30 *(Range: 0.15 to 0.45)* • PVS1_Supporting = 0.15 *(Range: 0 to 0.30)*	See categories at left
Overlap with ESTABLISHED benign genes or genomic regions	**2F**. Completely contained within an established benign CNV region.	−1	−1
	2G. Overlaps an established benign CNV, but includes additional genomic material.	0 (Continue evaluation)	0
Haploinsufficiency predictors	**2H**. Two or more HI predictors suggest that AT LEAST ONE gene in the interval is HI.	0.15	0.15
Section 3: Evaluation of gene number			
Number of protein-coding RefSeq genes wholly or partially included in the copy-number loss	**3A**. 0–24 genes	0	0
	3B. 25–34 genes	0.45	0.45
	3C. 35+ genes	0.90	0.90
Section 4: Detailed evaluation of genomic content using cases from published literature, public databases, and/or internal lab data ***(Skip to section 5 if either your CNV overlapped with an established HI gene/region in section 2, OR there have been no reports associating either the CNV or any genes within the CNV with human phenotypes caused by loss of function [LOF] or copy-number loss)***			
Individual case evidence—de novo occurrences	Reported proband (from literature, public databases, or internal lab data) has either: • A complete deletion of or a LOF variant within gene encompassed by the observed copy-number loss OR • An overlapping copy-number loss similar in genomic content to the observed copy-number loss AND…	See categories below	
	4A. …the reported phenotype is highly specific and relatively unique to the gene or genomic region,	Confirmed de novo: 0.45 points each Assumed de novo: 0.30 points each *(range: 0.15 to 0.45)*	0.90 (total)
	4B. …the reported phenotype is consistent with the gene/genomic region, is highly specific, but not necessarily unique to the gene/genomic region.	Confirmed de novo: 0.30 points each Assumed de novo: 0.15 point each *(range: 0 to 0.45)*	
	4C. …the reported phenotype is consistent with the gene/genomic region, but not highly specific and/or with high genetic heterogeneity.	Confirmed de novo: 0.15 point each Assumed de novo: 0.10 point each *(range: 0 to 0.30)*	
Individual case evidence—inconsistent phenotype	**4D**. …the reported phenotype is NOT consistent with what is expected for the gene/genomic region or not consistent in general.	0 points each *(range: 0 to −0.30)*	−0.30 (total)
Individual case evidence—unknown inheritance	**4E**. Reported proband has a highly specific phenotype consistent with the gene/genomic region, but the inheritance of the variant is unknown.	0.10 points each *(range: 0 to 0.15)*	0.30 (total)
Individual case evidence—segregation among similarly affected family members	**4F**. 3–4 observed segregations	0.15	0.45
	4G. 5–6 observed segregations	0.30	
	4H. 7 or more observed segregations	0.45	
Individual case evidence—nonsegregations	**4I**. Variant is NOT found in another individual in the proband's family AFFECTED with a consistent, specific, well-defined phenotype (no known phenocopies).	−0.45 points per family *(range: 0 to −0.45)*	−0.90 (total)
	4J. Variant IS found in another individual in the proband's family UNAFFECTED with the specific, well-defined phenotype observed in the proband.	−0.30 points per family *(range: 0 to −0.30)*	−0.90 (total)

Riggs ER et al.*Genet Med. 2020 Feb;22(2):245-257.*

実際の解釈論文：SEC１-5に分類

かなり煩雑！しかし有効利用可能
解釈実践だけでなく、
CNVの病原性判断における論理的かつ
深みのある理解が得られる

解釈医は一度は読み込むことを推奨

Table 1 continued

	4K. Variant IS found in another individual in the proband's family UNAFFECTED with the nonspecific phenotype observed in the proband.	−0.15 points per family *(range: 0 to −0.15)*	−0.30 (total)
Case–control and population evidence	**4L**. Statistically significant increase amongst observations in cases (**with a consistent, specific, well-defined phenotype**) compared with controls.	0.45 per study *(range: 0 to 0.45 per study)*	0.45 (total)
	4M. Statistically significant increase amongst observations in cases (**without a consistent, nonspecific phenotype OR unknown phenotype**) compared with controls.	0.30 per study *(range: 0 to 0.30 per study)*	0.45 (total)
	4N. No statistically significant difference between observations in cases and controls.	−0.90 (per study) *(range: 0 to −0.90 per study)*	−0.90 (total)
	4O. Overlap with common population variation.	−1 *(range: 0 to −1)*	−1
Section 5: Evaluation of inheritance pattern/family history for patient being studied			
Observed copy-number loss is de novo	**5A**. *Use appropriate category from de novo scoring section in section 4.*	*Use de novo scoring categories from section 4 (4A–4D) to determine score*	0.45
Observed copy-number loss is inherited	**5B**. Patient with **specific, well-defined** phenotype and no family history. CNV is inherited from an apparently unaffected parent.	−0.30 *(range: 0 to −0.45)*	−0.45
	5C. Patient with **nonspecific** phenotype and no family history. CNV is inherited from an apparently unaffected parent.	−0.15 *(range: 0 to −0.30)*	−0.30
	5D. CNV segregates with a consistent phenotype observed in the patient's family.	*Use segregation scoring categories from section 4 (4F–4H) to determine score*	0.45
Observed copy-number loss—nonsegregations	**5E**. *Use appropriate category from nonsegregation section in section 4.*	*Use nonsegregation scoring categories from section 4 (4I–4K) to determine score*	−0.45
Other	**5F**. Inheritance information is unavailable or uninformative.	0	0
	5G. Inheritance information is unavailable or uninformative. The patient phenotype is nonspecific, but is consistent with what has been described in similar cases.	0.10 *(range: 0 to 0.15)*	0.15
	5H. Inheritance information is unavailable or uninformative. The patient phenotype is highly specific and consistent with what has been described in similar cases.	0.30 *(range: 0 to 0.30)*	0.30

Only those CNVs otherwise meeting the reporting thresholds determined by your laboratory should be evaluated using this metric. See Supplemental Material 1 for a detailed description of each evidence category. Scoring: *pathogenic* 0.99 or more points, *likely pathogenic* 0.90 to 0.98 points, *variant of uncertain significance* 0.89 to −0.89 points, *likely benign* −0.90 to −0.98 points, *benign* −0.99 or fewer points.
CNV copy-number variant, *SVI* sequence variant interpretation, *UTR* untranslated region.

ガイドライン上のCNV解釈の枠組み

SEC1/SEC3: 属性

- CNV-LOSS or CNV-GAIN (解釈方法が異なる)
- サイズ・範囲・染色体バンド位置
- 内包(全長あるいは部分)する蛋白コード遺伝子や機能的重要領域の有無
- 内包(全長あるいは部分) する蛋白コード遺伝子数 (SEC.3)

SEC2 領域・遺伝子の病原性評価

- 確立したBenign領域の適用
- 確立した(or候補の)ハプロ不全(HI)/重複感受性(TS)領域や遺伝子の適用
- 確立した遺伝子or領域とのオーバーラップから推測される病原性判定

主としてCNV情報から知識データベース(ClinGen/DECIPHER等)を利用して判断

確立した病原性(Pathogenic/Benign)に比較的簡便・迅速にアクセスできる利便性

SEC2は比較的利用されている
それ以外はあまり使われていない

SEC4 文献を利用した関連患者＋家系情報

- 遺伝性や表現型の詳細情報

SEC5 当該患者＋家系情報

- 遺伝性や表現型の詳細情報

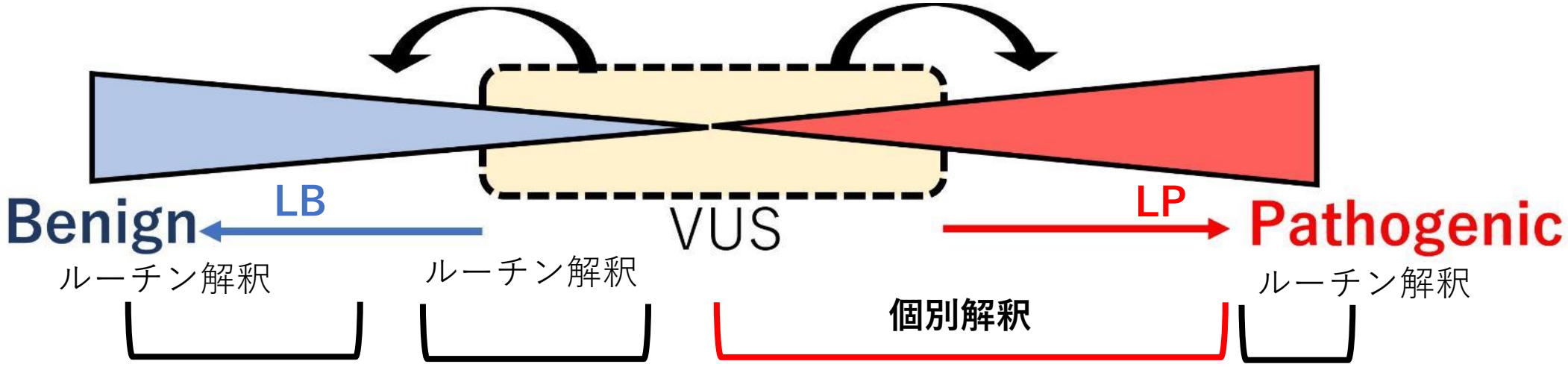
主として文献情報を用いる

遺伝性(家系情報)や表現型の評価も加わる

病原性判断の困難性・律速段階

病原性スペクトラムと解釈作業レベルとの関連

VUSの扱い：報告対象VUSか否かは重要
臨床的にはUPDATEが必要なVUSか否か
将来Pathogenic根拠が積み重なりそうか



確立されたDB情報利用
Bengin根拠
Pathogenicの根拠なし

DBのBenign根拠
Pathogenicの
候補なし

律速となる解釈領域
文献や当該症例での
遺伝型-表現型がKey

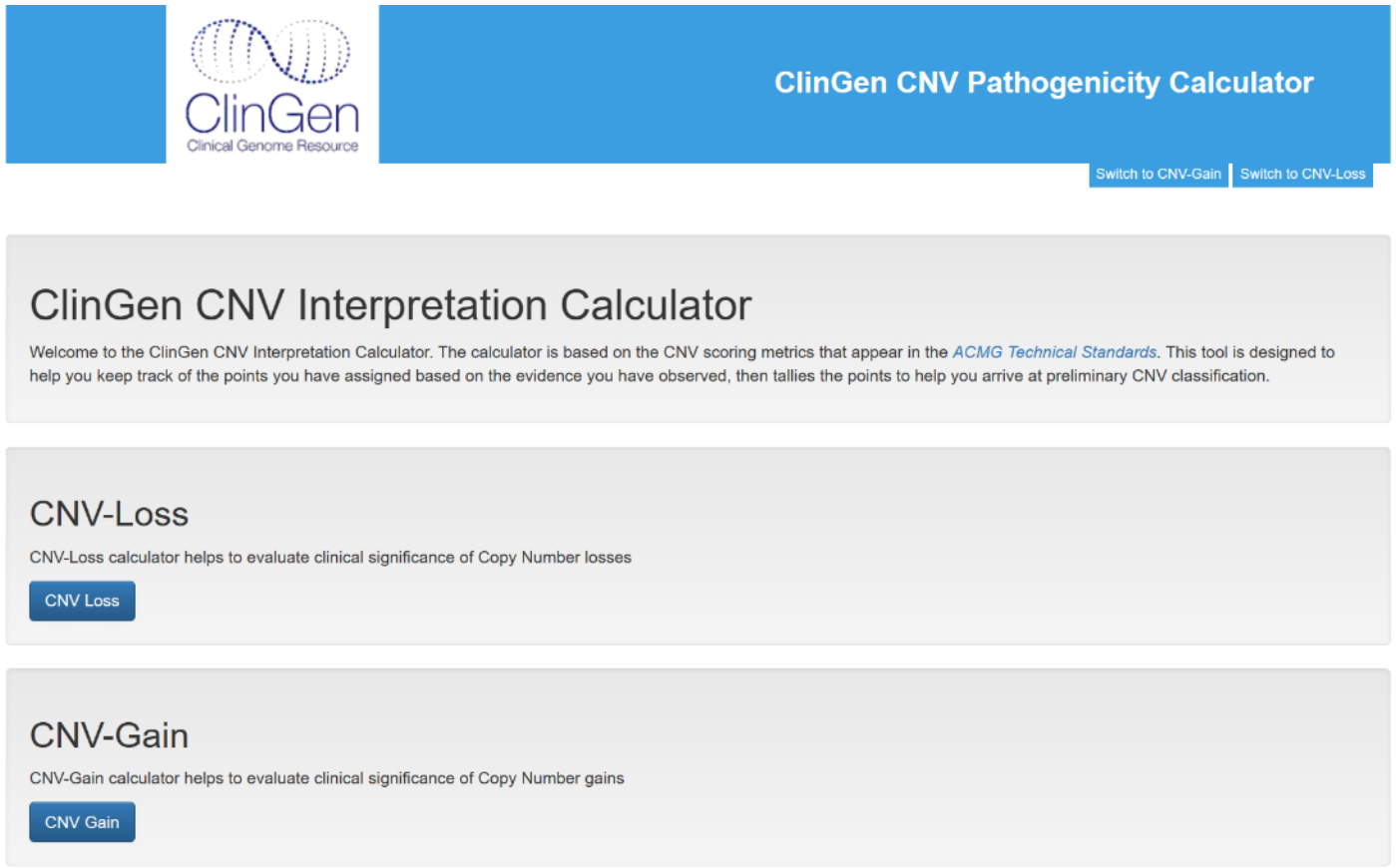
確立された
DB情報利用

報告対象外

報告対象（update必要なVUS含む）

ACMG/ClinGenガイドライン解釈Website（解釈自体はマニュアル：ポイント計算は自動）

https://cnvcalc.clinicalgenome.org/cnvcalc/

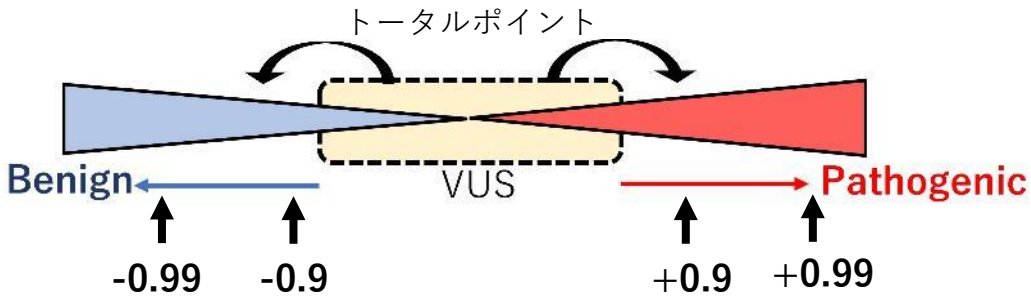


各チェック項目毎のポイント付与性
seq variantガイドラインとの整合性

Suggested CNV Point Value (Pathogenic/Benign)	Comparable ACMG/AMP Evidence Strength
0.90/-0.90	Very Strong
0.45/-0.45	Strong
0.30/-0.30	Moderate
0.15/-0.15	Supporting

トータルポイントと最終病原性評価

Proposed Interpretation	# Points
Pathogenic	0.99 or more
Likely Pathogenic	0.90 to 0.98
Uncertain	0.89 to -0.89
Likely Benign	-0.90 to -0.98
Benign	-0.99 or less



評価項目リストの一例

CNV解釈

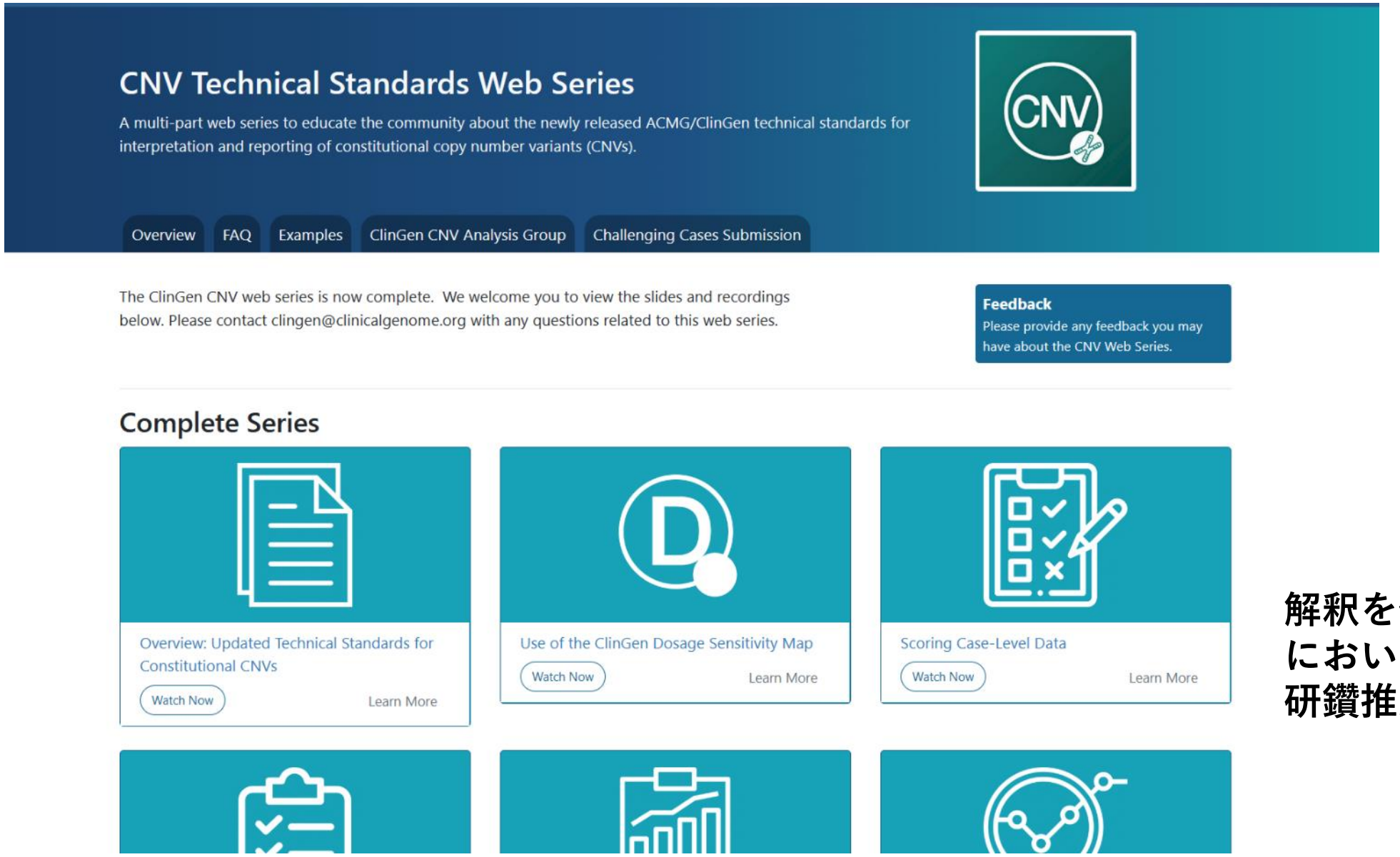
評価項目
CNV属性（GAIN or LOSS/Log2R）
CNVバンド位置（中間部/端部）
CNV内容 （上段minimum/下段maximum）
CNVサイズ（上段minimum/ 下段maximum）
Number of protein coding gene Number of OMIM morbid gene
確立した Benign CNV DGV gold standard or ClinGen HI/TS 40情報
その他候補Benign CNV(ClinGenDB等)
確立したPathogenic CNV ・ClinGen HI/TS score 3 ・DECIPHER CNV syndrome
確立したPathogenic Gene =Key gene（ClinGen HI/TS score 3 ）

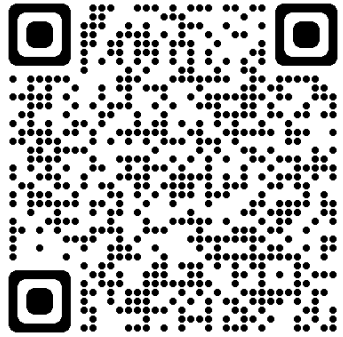
・ClinGen HI/TS score 2 (gene or region) ・OMIM morbid＋monoallelic遺伝子 ＋in silico 量感受性遺伝子
・ClinGen HI/TS score 1 (gene or region) ・OMIM＋in silico 両感受性遺伝子 ・OMIM morbid+monoallelic ・論文候補遺伝子
OMIM morbid＋ biallelic遺伝子 ＊症状との合致根拠強い場合
In-silico tool (X-CNV/varsome)
文献・DB症例における根拠
患者（家系）所見における根拠 (文献・DB症例根拠が優先)
病原性評価
診断評価 ①主要所見と関連する診断と遺伝子(領域) ②二次的・偶発的所見に関する診断と遺伝子 ③劣性遺伝保因者（基本は報告対象外）
追加検査に関するコメント 本人追加検査 両親・血縁者検査

ROH解釈

評価項目
LOH内容
LOHサイズ
染色体バンドレベル
当該染色体における インプリンティング領域と疾患
UPDの可能性
潜在性AR疾患の強い可能性
追加検査等コメント

ClinGen公開：CNV解釈トレーニングツール https://clinicalgenome.org/tools/cnv-webinar/





解釈を伝え、教育する立場においては本ツールでの研鑽推奨（私見）

ClinGen dosage sensitivity curation status

2023.9.9閲覧

- Total Curated numbers **3828**
- Total Curated genes **1537**
- Total Curated regions **514**

■Haploinsufficient **genes**, established (HI score3) **364** (23.7%)

■Haploinsufficient **regions**, established (HI score3) **48** (9.3%)

■Triplosensitive **genes**, established (TS score3) **2** (0.065%)

■Triplosensitive **regions**, established (TS score3) **22**(4.3%)

Established dosage sensitive genes/regionsのうち 94.5%がHI 5.5%がTS

共通設問

CNV症例

症例CNVの病原性評価とこれに応じた遺伝学的診断・その判断根拠を述べよ

・CNV病原性（5段階評価）　・遺伝学的診断　・判断根拠

ROH症例

症例ROHの臨床的判断(報告対象・報告対象外）とこれに応じた候補疾患・追加検査とその判断根拠を述べよ

・報告対象有無　・候補疾患＋追加検査　・判断根拠