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
症例２

静岡県立こども病院　清水健司

症例 2

0 歳女児：Pierre Robin 症候群、難聴

【CNV】chr7:95,167,037-97,905,676　　7q21.3　　log2ratio=-1.00 [Loss]

＊追加設問：当該 CNV と患者の臨床所見との関連について述べよ

7q21.3領域の2.73Mbの中間部欠失

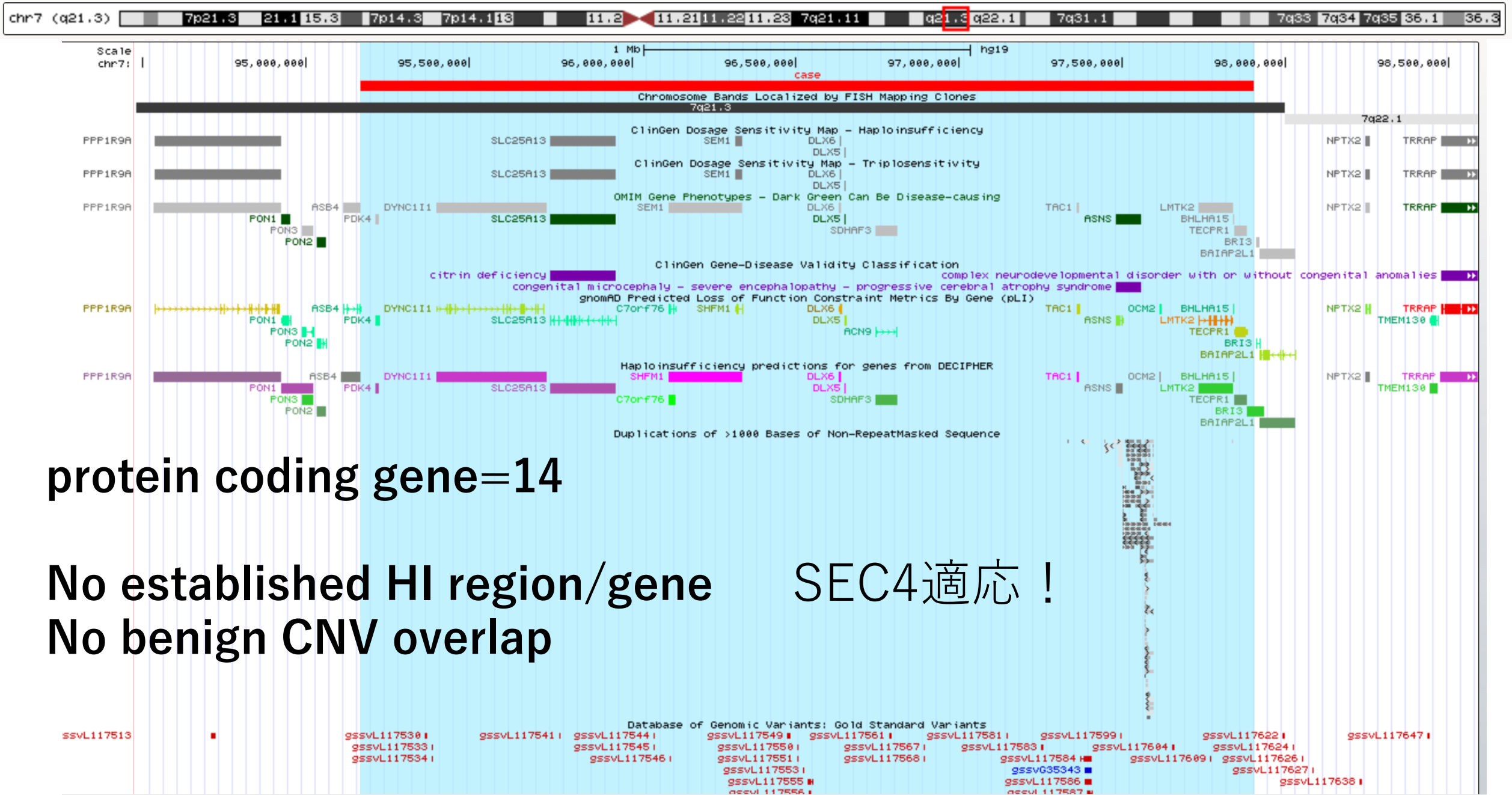
症例2　参加者解答（計7名中）とポイント

- 病原性評価：P: 2 人/LP: 2 人/ VUS: 2 人/ 不明 1 人
- 候補領域からの症例検索： 1 人
- 不完全浸透言及 ：2人

＊VUSとP/LPは明白な病原性判断の解離
Clinical Actionabilityが異なる！！

- 公開DBへの情報蓄積されるのを待つだけでなく、文献サーチで病原性を積み重ねる姿勢（教育的にも重要であり、患者への健康管理に直接役立つ知見も同時に得られる）

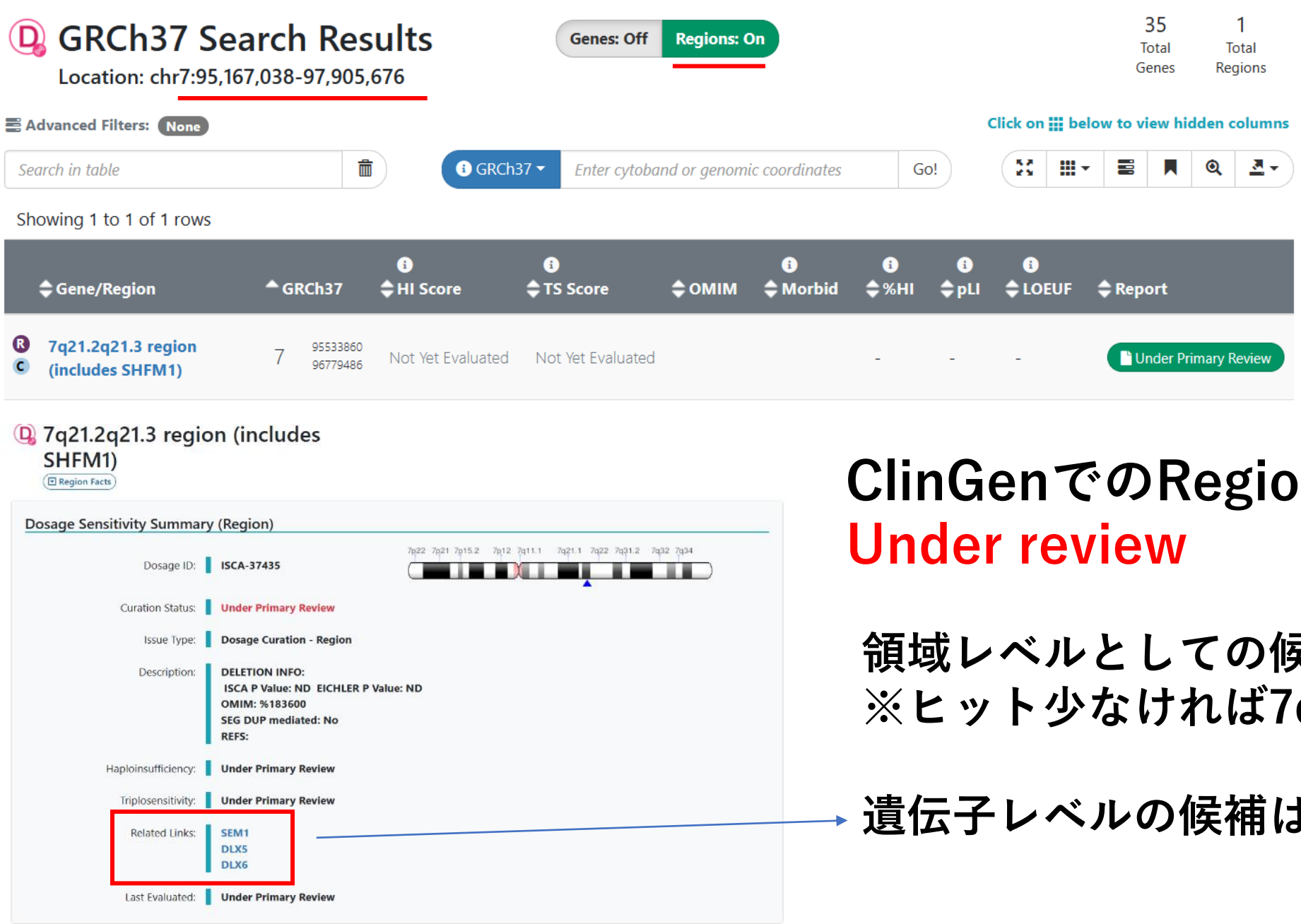
SEC2/3のルーチン評価



protein coding gene=14

No established HI region/gene　　SEC4適応！

No benign CNV overlap

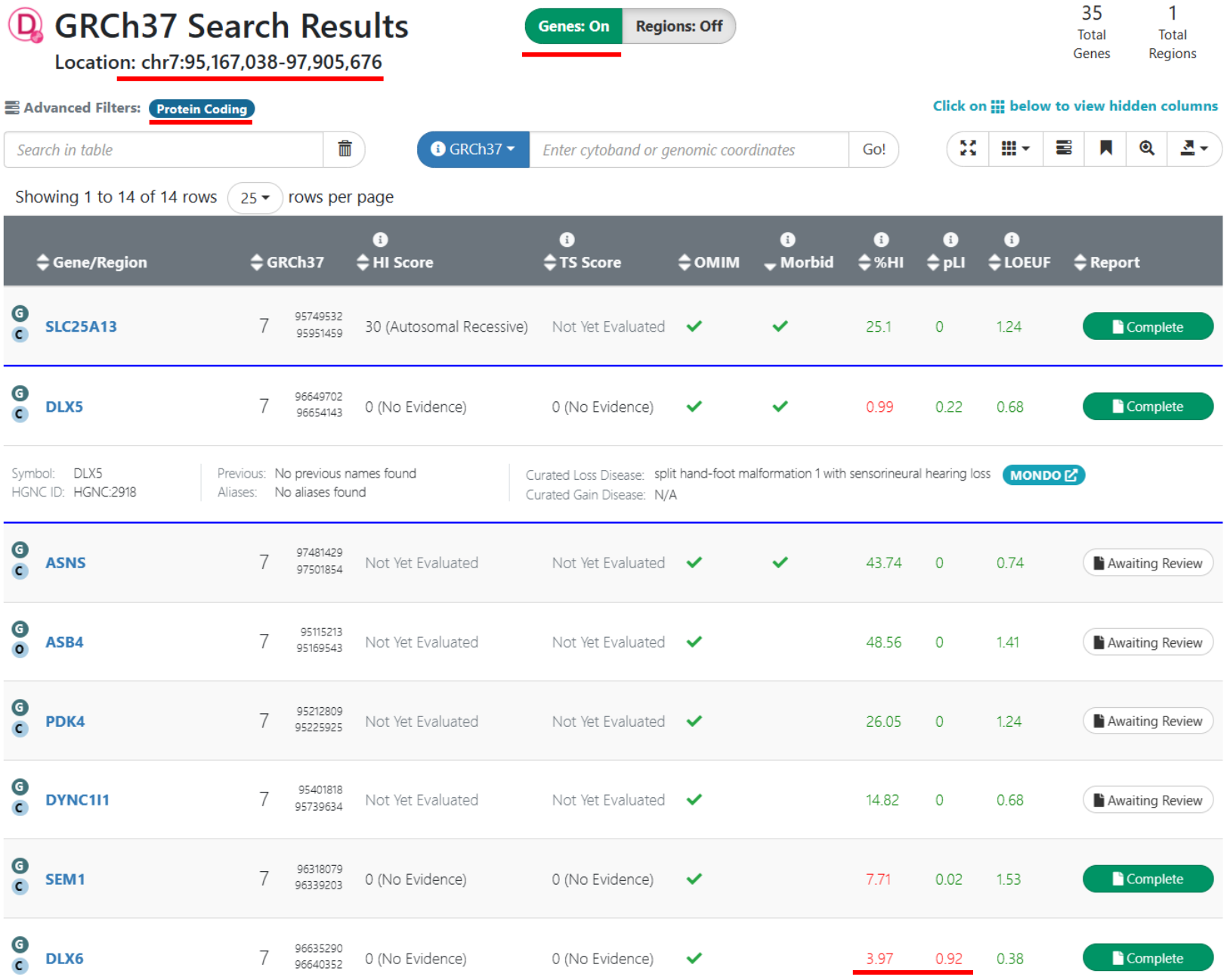


ClinGenでのRegion検索

Under review

領域レベルとしての候補： 7q21.3
※ヒット少なければ7q21と幅広く検索

遺伝子レベルの候補は？



候補遺伝子としては
ClinGen HI score 2(or1)
OMIM-morbid(AD)
In-Silico HI prediction(pLI/HI/LOEUF)

DLX5はOMIM morbid
(AD:**split hand-Foot malformation1**)として報告
*HIでの発症ではなさそう

DLX6=OMIM morbidではないが複数predictorでHIの評価
=0.15 pts（SEC2）

DLX5/DLX6が有力
（+SEM1=DSS1も）

183600

SPLIT-HAND/FOOT MALFORMATION 1; SHFM1

3遺伝子が絡む疾患か
→各遺伝子単独での機能喪失
症例はなし

Alternative titles; symbols

SPLIT-HAND/FOOT MALFORMATION 1 WITH OR WITHOUT DEAFNESS
SPLIT-HAND/FOOT DEFORMITY 1; SHFD1; SHSF1
SPLIT-HAND DEFORMITY
ECTRODACTYLY; ECD

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
7q21.3	Split-hand/foot malformation 1	183600	AD	3	DLX5	600028

Clinical Synopsis | Phenotypic Series | PheneGene Graphics

TEXT

A number sign (#) is used with this entry because some cases of split-hand/foot malformation-1 (SHFM1) represent a contiguous gene syndrome caused by deletion, duplication, or rearrangement of chromosome 7q21.3 involving the DSS1 (601285), DLX5 (600028), and DLX6 (600030) genes and possible regulatory elements in the region. Evidence exists that SHFM1 can also be caused by heterozygous mutation in the DLX5 gene.

DSS1＝SEM1

cytoband level検索

7q21.3　AND
（deletion OR haploinsufficiency OR loss OR monosomy OR Split-Hand/Foot Malformation）

candidate gene level検索

DLX5　AND
（deletion OR haploinsufficiency OR loss OR monosomy OR Split-Hand/Foot Malformation）

DLX6　AND
（deletion OR haploinsufficiency OR loss OR monosomy OR Split-Hand/Foot Malformation）

（DSS1 OR SEM1）　AND
（deletion OR haploinsufficiency OR loss OR monosomy OR Split-Hand/Foot Malformation）

1 Cite Share

Split Hand/Foot Malformation Associated with **7q21.3** Microdeletion: A Case Report.

Sivasankaran A, Srikanth A, Kulshreshtha PS, Anuradha D, Kadandale JS, Samuel CR.

Mol Syndromol. 2016 Feb;6(6):287-96. doi: 10.1159/000443708. Epub 2016 Feb 3.

PMID: 27022330　Free PMC article.

Split hand/foot malformation (SHFM) or ectrodactyly is a rare genetic condition affecting limb development. ...FISH using locus-specific BAC probes confirmed the microdeletion of **7q21.3**. Chromosomal microarray analysis also revealed a mic ...

2 Cite Share

Phenotypic subregions within the **split-hand/foot malformation** 1 locus.

Rasmussen MB, Kreiborg S, Jensen P, Bak M, Mang Y, Lodahl M, Budtz-Jørgensen E, Tommerup N, Tranebjærg L, Rendtorff ND.

excellent review

Hum Genet. 2016 Mar;135(3):345-57. doi: 10.1007/s00439-016-1635-0. Epub 2016 Feb 2.

PMID: 26839112

Split-hand/foot malformation 1 (SHFM1) is caused by chromosomal aberrations involving the region **7q21.3**, DLX5 mutation, and dysregulation of DLX5/DLX6 expression by long-range position effects. ...We report on a new family with five affec ...

3 Cite Share

7q21.3 Deletion involving enhancer sequences within the **gene** DYNC1I1 presents with intellectual disability and **split hand-split foot malformation** with decreased penetrance.

Delgado S, Velinov M.

Mol Cytogenet. 2015 Jun 13;8:37. doi: 10.1186/s13039-015-0139-2. eCollection 2015.

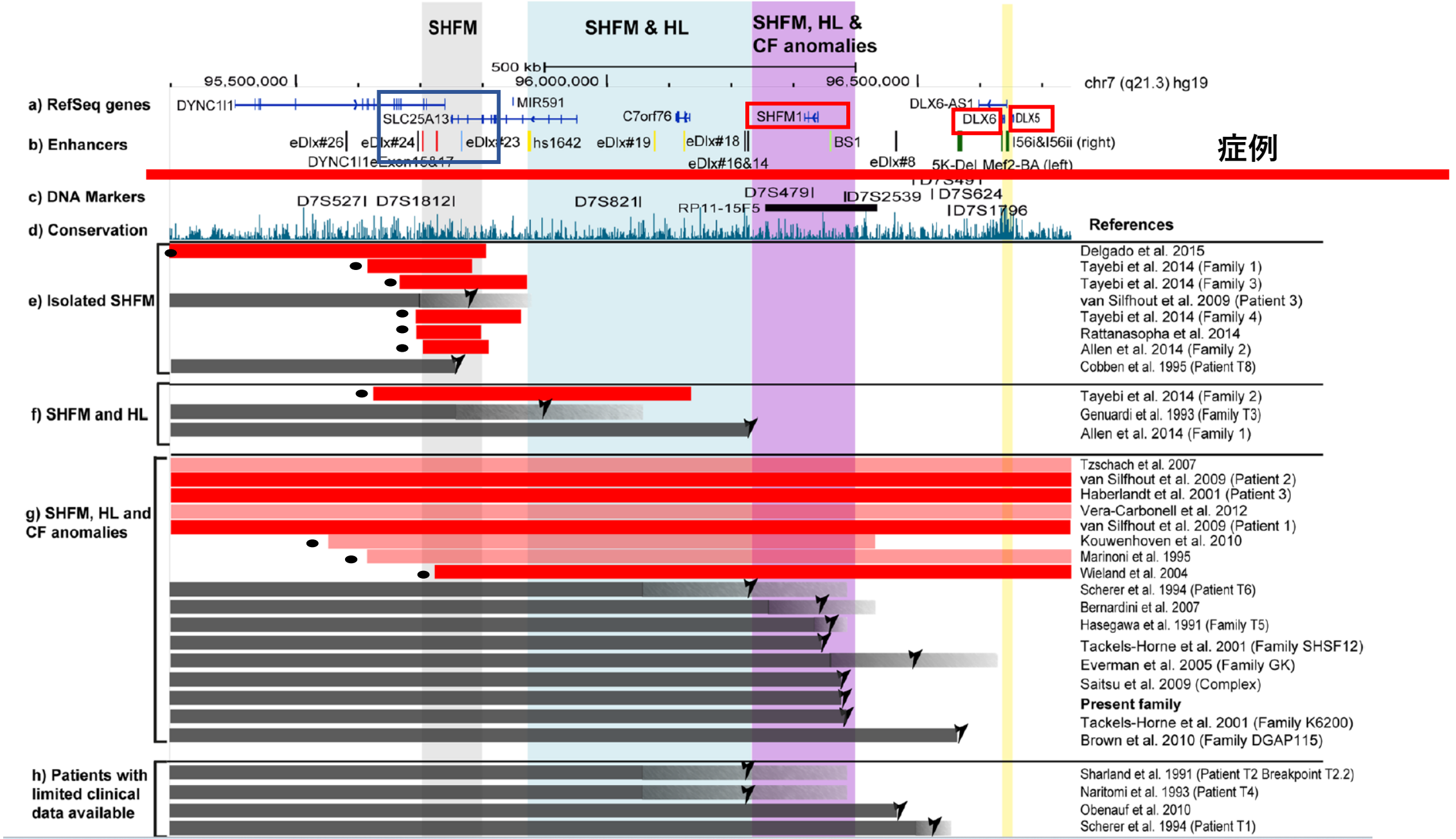
PMID: 26075025　Free PMC article.

Split hand-split food **malformation** (SHFM) is a **congenital** defect of limb development that involves the central rays of the autopod and presents with median clefts of the hands and feet. ...The **deleted** region is located proximally (centrom ...

4

Array comparative genomic hybridization characterization of multiple interstitial **deletions** involving 7p22.1, 7q11.23, **7q21.3**-q22.1, 19p13.**3**-p12, and 19q13.11-

当該CNVに包含される複数CNV



supplmentary material

0.15 pts

0.15 pts

Aberration type		Deletion	Deletion
SHFM1 case report		*Patient reported by Marinoni et al. 1995*	Patient reported by Wieland et al. 2004
Aberration-type and breakpoint positions	Aberration	46,XY,del(7)(q21.2q22.1)dn	A novel 0.9-1.8 Mb microdeletion at 7q21.3
	Level of mapping and breakpoint positions at chr7q21.3 (hg19)	Analysis of microsatellite markers found deletion of the paternal allele of D7S527, D7S479 and D7S554, maximal size 1.7 Mb (chr7:95615066-97325191)	Microsatelite and Southern blot mapped the proximal breakpoint between DYNC1I1 and D7S821 (chr7:95727736-96057501), and the distal breakpoint between DLX5 and D7S618 (chr7:96654143-97318664), maximal range 1.6 Mb (chr7:95727736-97318664), with loss of paternal markers D7S821, D7S491 and D7S624
	Position of additional breakpoints (hg19)		
Number of patients	Patients *including obligate carriers* /Affected patients	1/1	1/1
SHFM1 manifestations	SHFM manifestations	Yes	Yes
	Hearing loss (HL)	No (appeared to hear well at age 28 months)	Yes
	Craniofacial (CF) anomalies	Yes	Yes
Other manifestations	Other manifestations	Feeding difficulties, failure to thrive. Arched eyebrows, small, triangular nose, depressed nasal bridge, prominent antitragus, overfolded helices, attached earlobes, hypotonia	Inner ear malformation with Modini dysplasia
	Developmental delay	Global developmental delay, no discernible words at age 28 months	Psychomotor development normal at age four years
	Neuropsychiatric	Not described	Not described

DLX5, DLX6, SEM1 を含む
類似CNV loss症例　2人
specific(SHFM+α)かつ
assumed de novo
→0.15x2 pt

Aberration type		Deletion	Deletion	Deletion	Deletion
SHFM1 case report		*Patient reported by Kouwenhoven et al. 2010*	*Family 1 reported by Tayebi et al. 2014*	*Family 2 reported by Tayebi et al. 2014*	*Family reported by Delgado et al. 2015*
Aberration-type and breakpoint positions	Aberration	A novel 880 kb microdeletion at 7q21.3	A 167 kb microdeletion at 7q21.3	A 510 kb microdeletion at 7q21.3	A 1.031.663 bp deletion at 7q21.3
	Level of mapping and breakpoint positions at chr7q21.3 (hg19)	Ultra high CGH refined the microdeletion to 880 kb (chr7:95552066–96432064)	Array CGH and PCR of breakpoints. Genomic position: chr7:95615187-95783313	Array CGH and PCR of breakpoints. Genomic position: chr7:95624825-96135521	Oligo SNP array. Genomic position: chr7:94769383-95801045
	Position of additional breakpoints (hg19)				
Number of patients	Patients *including obligate carriers* /Affected patients	1 / 1	3 / 3	5 / 5	4/3
SHFM1 manifestations	SHFM manifestations	Yes	Yes	Yes	Yes (n=3, variable expression)
	Hearing loss (HL)	Yes (developed after publication according to Tayebi et al. 2014)	No	Yes	No
	Craniofacial (CF) anomalies	No	No	No	No
Other manifestations	Other manifestations	No	No	No	Not described
	Developmental delay	Development within normal limits at age 2 years	No	No	Developmental delay (n=4) and speech delay (n=3)
	Neuropsychiatric	No	No	No	Aggressive behaviour and adjustment disorder (n=1); Mood disorder (n=1)

DLX5, DLX6遺伝子の調節候補領域を含む類似CNV loss症例

１人
specific(SHFM1+α)
＋assumed de novo
→0.15pt

その他３家系
7 or more segregations
→0.45pt

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—segregation among similarly affected family members	**4F**. 3–4 observed segregations	0.15
	4G. 5–6 observed segregations	0.30
	4H. 7 or more observed segregations	0.45

SEC4　Case-level data文献・データベース情報を「再現性・客観性をもって」病原性判断としていかに積み上げるか

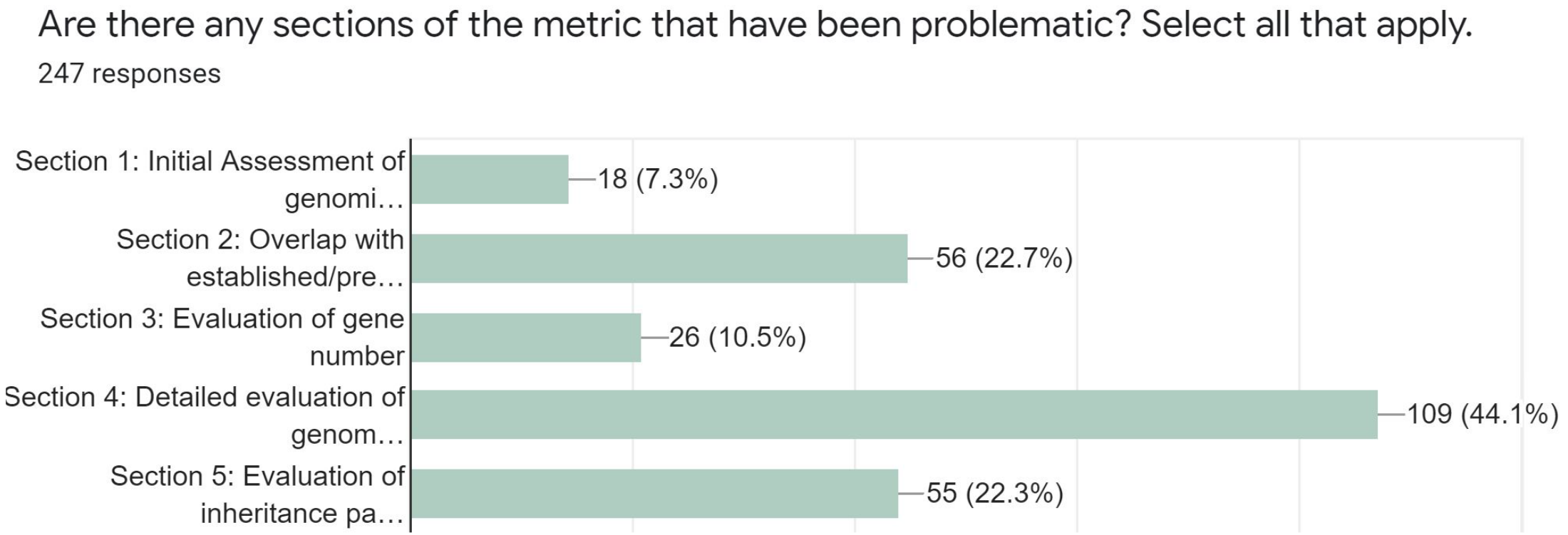
0.15(predicted HI gene)+0.9(published literatureによる包含CNV症例)＝**1.05 pts**

CNV Technical Standards Pre-Webinar Survey Results

https://clinicalgenome.org/site/assets/files/5411/cnv_webinar_survey_results.pdf

Responses as of 11/9/2020

ClinGenによるアンケート調査



SEC４が難しい

CNV Technical Standards Pre-Webinar Survey Results

https://clinicalgenome.org/site/assets/files/5411/cnv_webinar_survey_results.pdf

Responses as of 11/9/2020

SEC 4 のハンドリング

Are there any sections of the metric that have been problematic?
Free text responses

- 4 individuals reported that section 4 can be time consuming and subjective
- 11 individuals stated relevant literature can be tough to obtain and apply to the criteria
- 3 individuals requested the best resources to use for curation
- 5 individuals reported increased numbers of VUS classifications and difficulty getting genes to other classifications
- 1 individual asked which guidelines to use for CNVs with low penetrance
- 3 individuals reported that they had difficulty with X chromosome CNVs

当該患者情報の利用（SEC5）

指趾所見の記載なし

→文献情報（SEC4）疾患名Split Hand-Foot Malformation1 :SHFM1がメインの所見とした病名ではあるが、SHFM含む合併症は各々**不完全浸透**である。本症例では他のSHFM1のmain featuresであるcraniofacial phenotypeとhearing loss（＋developmental delay)は合併。

★SHFMがあるかどうかは**もう一度詳細な指趾所見の診察**を要する

しかし所見がない場合も十分考えられ、現時点でこの2つの組み

合わせだけでの特異性は乏しく、nonspecific but consistentと判定　**0.1 pts**

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

合計　0.15(predicted HI gene)+0.9(published literature)+0.1(patient information)＝**1.15pts**

CNV病原性：Pathogenic　　**遺伝学的診断：7q21.3 deletion including SHFM1 critical region**

SEC4/SEC5:　臨床的特異性の把握とCNV病原性判断への寄与

①relatively **unique**

その原因にほぼ特異的→ 下口唇小窩(*IRF6* gene)/
alpha-galactosidase A 欠損（*GLA* gene)

②highly **specific,** not unique

疾患スペクトラム→ 両側小眼球 / 肥大型心筋症 /特徴的外表所見

③**non-specific** but **consistent**

一般頻度高くcommonな所見→ 知的障害/ 自閉スペクトラム症

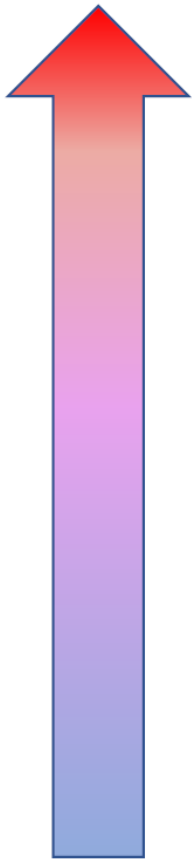
既報患者表現型の把握
当該患者表現型の記録

↓

病原性評価の蓄積に寄与＋健康管理に直結

ClinGen Webinar Presentation:Question & Answers

Question 4: How do I determine which phenotype category to use?



Highly specific, relatively unique

- Use rarely
- Use when you are **confident** that your variant includes the correct gene
  - Pathognomonic features
  - Biochemical confirmation
  - Caused by a small number of genes, other causes ruled out

Highly specific, not necessarily unique

- More distinct, less commonly observed phenotypes
- Phenotypes with less genetic heterogeneity and/or less potential for non-genetic etiologies
- Not confident that your gene is the only potential cause

Non-specific and/or high genetic heterogeneity

- Use more commonly
- Developmental delay, intellectual disability, seizures NOS, autism
- **When in doubt, use this category**

- There is currently no specific threshold to determine this
  - Excellent project idea!
- Use your clinical judgement
  - How sure are you that the phenotype you are given is accurate (e.g., autism)?
  - How sure can you be that you are in the correct gene?
- When in doubt, score in the most conservative category

症例2の注目要素

A. Pathogenic classification of CNV 遺伝学的影響（CNV領域と内包遺伝子の観点）による病原性評価分類			ポイント ＊"確立した"というのはClinGen HIもしくはTS scoreで３であること
	A1	recurrent common deletion/duplicatoin	LCRに起因する非アレル間相同組換え(NAHR)によるメジャー(common)で病原性確立したCNV
	A2	recurrent subtype deletion/duplicatoin	LCR組換えによる複数領域のrecurrent CNVが集積している領域のsubtype CNV（病原性確立）
	A3	non-recurrent deletion/duplicatoin including established critical gene(s)/region	A1/A2のメカニズムと異なり、切断点が共通でなく（nonhomologous end joining:NHEJ等のメカニズム） 内包する病原性確立した遺伝子（領域）を持つCNV
	A4	other1 (partial overlap deletion/duplicatoin of established critical gene/region)	内包する病原性確立した遺伝子(領域）を完全に含まず、一部のみが重なる ①CNV断端の一方が遺伝子(領域)内に存在する場合 ②CNVの両端が遺伝子(領域)内に存在（いわゆるintragenic CNV） ＊①におけるduplicationの評価はさらに複雑
	A5	other2 (non-established deletion/duplicatoin literature-search based)	CNVに内包される確立した遺伝子(領域)が明らかでないため、文献もしくはDBレベルでの症例根拠の蓄積が必要な場合