

マイクロアレイ染色体 (CMA) 検査の 結果解釈に必要なデータベースの 使用方法

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Webinarの構成

- イントロダクション
 - マイクロアレイ染色体検査 (CMA) 検査 超入門
 - BEDファイル形式データの取扱い
 - マイクロアレイ染色体検査の結果解釈の補助ソフトウェアツール (CAS) の使用方法
 - マイクロアレイ (CMA) 検査の結果解釈に必要なデータベースの使用方法
- 実践編
 - 解析例 1 : CASを使用して疾患関連性を調べたLossの例
 - 解析例 2 : CASと各種データベースを使用して疾患関連性を調べたLossの例
 - 解析例 3 : CASと各種データベースを使用して疾患関連性を調べたGainの例
 - 解析例 4 : 疾患関連性領域近傍にあるBenignのLossの例
- Advanced編
 - データベース「UCSC」使用方法
 - 解析例 5 : UCSCを用いて、Uncertain Significance と推定される例
 - インプリンティング疾患の解釈について

CMAの結果とその内容

- ① どの領域に : 染色体領域、ゲノムポジション
- ② なにが : コピー数低下、コピー数上昇

| ゲイン/ロス領域表 Gain/Loss Intervals Table | | | | | |
|--|--|--|--|---------------------|--|
| 染色体 Chr | 最小始点-終点(bp) 最大始点-終点(bp) Min Start-Stop(bp) Max Start-Stop(bp) | 最小サイズ(kb) 最大サイズ(kb) Min Size(kb) Max Size(kb) | 最小サイトバンド 最大サイトバンド Min Cytoband Max Cytoband | ゲイン/ロス Gain/Loss | アノテーション Annotations |
| chr1 | 746608-3375421 647288-3404363 | 2,628.814 2,757.076 | p36.33 - p36.32 p36.33 - p36.32 | 0.564656 | FAM87B, LINC00115, LINC01128, FAM41C, LINC02593, SAMD11, NOC2L, KLHL17, PLEKHN1, PERM1, HES4, ISG15, AGRN, LOC100288175, RNF223, C1orf159, LINC01342, MIR200B, MIR200A, MIR429, TTLL10, TNFRSF18, TNFRSF4, SDF4, B3GALT6, C1QTNF12, UBE2J2, SCNN1D, ACAP3, MIR6726, SNORD167, PUSL1, INTS11, MIR6727, CPTP, TAS1R3, DVL1, MIR6808, MXRA8, AURKAIP1, CCNL2, MRPL20-AS1, MRPL20, ANKRD65, TMEM88B, LINC01770, VWA1, ATAD3C, ATAD3B, ATAD3A, TMEM240, SSU72, FNDC10, LOC105378586, MIB2, MMP23B, MMP23A, CDK11B, SLC35E2B, CDK11A, SLC35E2A, NADK, GNB1, CALML6, TMEM52, CFAP74, GABRD, LOC105378591, PRKCZ, PRKCZ-AS1, FAAP20, SKI, MORN1, LOC100129534, RER1, PEX10, PLCH2, PANK4, HES5, TNFRSF14-AS1, TNFRSF14, LOC100996583, PRXL2B, MMEL1, TTC34, ACTRT2, PRDM16-DT, PRDM16, MIR4251, ARHGEF16 |

①

②

CMAの結果解釈に必要な理解

- **ハプロ不全 (Haploinsufficiency: HI)**
領域や遺伝子が欠失することによる疾患発症
- **重複感受性 (Triplosensitivity: TS)**
領域や遺伝子が重複することによる疾患発症

ハプロ不全 (HI) ①

➤ Haploinsufficiency Score (HI Score)

ClinGen (Clinical Genome Resource) が提唱する
ハプロ不全の指標

| HI/TS Score | Description | Predicted Pathogenicity |
|-------------|--|----------------------------|
| 3 | Sufficient evidence | Pathogenic |
| 2 | Emerging evidence | Likely Pathogenic |
| 1 | little evidence | Uncertain |
| 0 | no evidence | Uncertain or Likely Benign |
| 40 | Dosage sensitivity unlikely | Benign |
| 30 | Gene associated with autosomal recessive phenotype | |

ハプロ不全 (HI) ②

➤ pLI, LOEUF

WESやWGSデータから算出されたHIの指標

➤ %HI

文献やCNVデータなどを基にしたHIの指標

HIを示唆するCut off値

- $pLI \geq 0.9$
- $LOEUF < 0.35$
- $\%HI \leq 10\%$

pLI, LOEUF, %HIはHIを確定するものではない 

重複感受性 (TS)

➤ Triplosensitivity Score (TS Score)

ClinGen (Clinical Genome Resource) が提唱する
重複感受性の指標

| HI/TS Score | Description | Predicted Pathogenicity |
|-------------|--|----------------------------|
| 3 | Sufficient evidence | Pathogenic |
| 2 | Emerging evidence | Likely Pathogenic |
| 1 | little evidence | Uncertain |
| 0 | no evidence | Uncertain or Likely Benign |
| 40 | Dosage sensitivity unlikely | Benign |
| 30 | Gene associated with autosomal recessive phenotype | |

HIの適用対象

| HI関連データ | 遺伝子 | 領域 |
|--------------------|-----|----|
| HI Score (ClinGen) | ○ | ○ |
| pLI, LOEUF | ○ | × |
| %HI | ○ | × |

TSの適用対象

| TS関連データ | 遺伝子 | 領域 |
|--------------------|----------------------|----|
| TS Score (ClinGen) | ○ | ○ |
| | <i>LMNB1, PLP1のみ</i> | |

※pLI, LOEUF, %HIと対比可能なTS関連データはない

HI, TSの検討に有用なデータベース

➤ DECIPHER

➤ ClinGen

➤ UCSC Genome Browser

DECIPHER

Databases of genomic variation and Phenotype
in Humans using Ensembl Resources

DECIPHER About Browse ▾ DDD (UK) Search DECIPHER Help Join Log in →

Mapping the clinical genome

Explore DECIPHER

It's free and you don't need to log in

DECIPHER is used by the clinical community to share and compare phenotypic and genotypic data. The DECIPHER database contains data from 42,729 patients who have given consent for broad data-sharing; DECIPHER also supports more limited sharing via consortia. [Have a look at the numbers.](#)

Anyone can browse publicly-available patient data on DECIPHER and request to be put in contact with the responsible clinician. Why? [Because sharing benefits everyone.](#)

[Explore DECIPHER's genome browser](#)

[Delve into the Human Phenotype Ontology](#)

[Search all open-access DECIPHER data](#)

Join DECIPHER

Be part of the sharing community

Projects affiliated to DECIPHER can deposit and share patients, variants, and phenotypes to invite collaboration and facilitate diagnosis. Once deposited, you can use DECIPHER to identify and prioritise potential matches, and you can request notifications as soon as new matches arrive.

As well as influencing individual patient outcomes, use of DECIPHER has contributed to over [2600 published articles since 2004](#). It's still free, and you are in control of what data to make public.

[Join now](#)

[Find out more](#)

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Email address

Password

[Log in](#)

[Reset your password](#)

<https://www.deciphergenomics.org/> (2022.5.2アクセス)

Mapping the clinical genome

- 赤枠に検出されたCNV領域を入力する
(例) BEDファイルのデータ

22 18912231 21465672 **Loss**

入力方法

検出したCNVの染色体番号

“grch37:22:18912231-21465672”と記入

↓
使用した標準配列

↓
CNVのGenome position

Search results for "grch37:22:18912231-21465672" GRCh38 22:18924718-21111383 (85% match) [\(Refine Search\)](#)

Patient variants 1222

CNV syndrome variants 2

DDD research variants 15

Genes 82

Results

Browser

Syndrome Variants: 1 to 2 of 2

Filter...

| Syndrome | Location | Genotype | Variant Class | Size | Phenotype(s) |
|--|--|--------------|-----------------|---------|--|
| 22q11 deletion syndrome (Velocardiofacial / DiGeorge syndrome) | 22 ¹⁹⁰²²²⁷⁹ ₂₁₀₉₈₁₅₆ GRCh38 | Heterozygous | Deletion | 2.08 Mb | Abnormality of the heart, Delayed speech and language development, Hypocalcemia, Nasal speech, T lymphocytopenia |
| 22q11 duplication syndrome | 22 ¹⁹⁰²²²⁷⁹ ₂₁₀₉₈₁₅₆ GRCh38 | Heterozygous | Duplication | 2.08 Mb | Intellectual disability, Nasal speech, Telecanthus |

CNV syndrome variantsのResultsに22q11.2 deletion syndromeの表示
22q11.2欠失症候群

※下段は22q11.2 duplication syndrome
22q11.2重複症候群

**欠失・重複を
間違えない！！**

Search results for "grch37:22:18912231-21465672" GRCh38 22:18924718-21111383 (85% match) [\(Refine Search\)](#)

Patient variants **1226**

CNV syndrome variants **2**

DDD research variants **15**

Genes 82

Genes: 21 to 30 of 82

Show: All genes ▾

Filter...

| Name / Description | Location | pLI | LOEUF | sHet | %HI | GenCC | OMIM / Morbid | G2P | ClinGen | Links |
|--|-------------------------|------|-------|-------|-------|----------------------------|--------------------|----------------------|---|----------------------|
| FAM230G family with sequence similarity 230 member G | 22 20338805 20354372 | 0.40 | 1.69 | - | - | - | - | - | - | View |
| FAM246C family with sequence similarity 246 member C (gene/pseudogene) | 22 19029524 19031242 | - | - | - | - | - | - | - | - | View |
| GNB1L G protein subunit beta 1 like | 22 19783223 19854939 | 0.00 | 1.05 | 0.005 | 48.56 | - | OMIM | - | - | View |
| GP1BB glycoprotein Ib platelet subunit beta | 22 19723539 19724771 | 0.51 | 1.33 | - | 78.00 | Definitive: Supportive: | 1 2 OMIM Morbid | - | Definitive: AR Haploinsufficiency: 30 | View |
| GSC2 goosecoid homeobox 2 | 22 19146993 19150292 | 0.00 | 1.91 | - | 65.74 | - | OMIM | - | - | View |
| HIRA histone cell cycle regulator | 22 19330698 19447450 | 1.00 | 0.14 | 0.380 | 19.47 | - | OMIM | Limited: Monoallelic | Haploinsufficiency: 0 Triplosensitivity: 0 | View |

Geneのタブでは各遺伝子のHI/TS関連データを表示

ClinGen: Dosage Sensitivity

Clinical Genome Resource



Get Started About Us▼ Curation Activities▼ Working Groups▼ Expert Panels▼ Documents & Announcements Tools 🔍

Gene▼ Enter a gene symbol or HGNC ID (Examples: ADNP, HGNC:15766) Search

All Curated Genes Gene-Disease Validity▼ Dosage Sensitivity▼ Clinical Actionability▼ Curated Variants▼ Statistics Downloads More▼ ?▼

GRCh37 Search Results

Location: chr22:18912231-21465672

Genes: On Regions: On

108 Total Genes
8 Total Regions

Advanced Filters: None

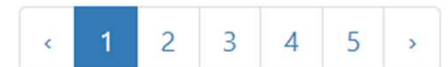
Click on below to view hidden columns

Search in table

GRCh37▼ chr22:18912231-21465672 Go!



Showing 1 to 25 of 116 rows 25▼ rows per page



chr22:18912231-21465672と入力して”Go!”をクリック
(染色体番号のところに”chr”を必ず入れる。)

<https://search.clinicalgenome.org/kb/gene-dosage?page> (2022.5.7アクセス)

Gene Enter a gene symbol or HGNC ID (Examples: ADNP, HGNC:15766) Search

All Curated Genes Gene-Disease Validity▼ Dosage Sensitivity▼ Clinical Actionability▼ Curated Variants▼ Statistics Downloads More▼ ?▼

GRCh37 Search Results

Genes: On Regions: On

108 Total Genes
8 Total Regions

Location: chr22:18912231-21465672

領域に含まれる遺伝子 + HI/TS関連データのある領域
がすべて表示される

Click on  below to view hidden columns













Showing 1 to 1 of 116 rows 25 rows per page

1 2 3 4 5

| Gene/Region | GRCh37 | HI Score | TS Score | OMIM | Morbid | %HI | pLI | LOEUF | Report |
|--|-------------------------|-------------------------|-------------------------|------|--------|-------|-----|-------|-----------------|
|  PRODH | 22 18900287 18924066 | Not Yet Evaluated | Not Yet Evaluated | ✓ | ✓ | 45.38 | 0 | 1.1 | Reopened |
|  22q11.2 recurrent (DGS/VCFS) region (proximal, A-B) (includes TBX1) | 22 18912231 20287208 | 3 (Sufficient Evidence) | 3 (Sufficient Evidence) | | | - | - | - | Complete |
|  22q11.2 recurrent (DGS/VCFS) region (proximal, A-D) (includes TBX1) | 22 18912231 21465672 | 3 (Sufficient Evidence) | 3 (Sufficient Evidence) | | | - | - | - | Complete |
|  DGCR5 | 22 18958011 19018755 | Not Yet Evaluated | Not Yet Evaluated | ✓ | | - | - | - | Awaiting Review |
|  LOC100287576 | 22 18985531 18987264 | -1 (Pseudogene) | -1 (Pseudogene) | | | - | - | - | Not Reviewable |

GenesをクリックしてoffにするとRegionのみの表記となる

Showing 1 to 8 of 8 rows

| Gene/Region | GRCh37 | HI Score | TS Score | OMIM | Morbid | %HI | pLI | LOEUF | Report |
|--|----------------------|----------------------------------|-------------------------|------|--------|-----|-----|-------|---------------------|
| R 22q11.2 recurrent (DGS/VCFS) region (proximal, A-B) (includes TBX1) C | 22 18912231-20287208 | 3 (Sufficient Evidence) | 3 (Sufficient Evidence) | | | - | - | - | Complete |
| R 22q11.2 recurrent (DGS/VCFS) region (proximal, A-D) (includes TBX1) C | 22 18912231-21465672 | 3 (Sufficient Evidence) | 3 (Sufficient Evidence) | | | - | - | - | Complete |
| R 22q11.21 population region (gnomAD-SV_v2.1_DEL_22_181446) C | 22 20366424-20371500 | 40 (Dosage Sensitivity Unlikely) | 0 (No Evidence) | | | - | - | - | |
| R 22q11.21 population region (DGV_Gold_Standard_June_2021_gssvL76981) C | 22 20428070-20499197 | 40 (Dosage Sensitivity Unlikely) | 0 (No Evidence) | | | - | - | - | Complete |
| R 22q11.21 population region (gnomAD-SV_v2.1_DEL_22_181452) C | 22 20451599-20457600 | 40 (Dosage Sensitivity Unlikely) | 0 (No Evidence) | | | - | - | - | Complete |
| R 22q11.21 population region (gnomAD-SV_v2.1_DEL_22_181459) C | 22 20693999-20699000 | 40 (Dosage Sensitivity Unlikely) | 0 (No Evidence) | | | - | - | - | Complete |
| R 22q11.2 recurrent region (central, B/C-D) (includes CRKL) C | 22 20731986-21465672 | 2 (Emerging Evidence) | 1 (Little Evidence) | | | - | - | - | Complete |
| R 22q11.2 recurrent region (central, C-D) (includes CRKL) C | 22 21092338-21465672 | Not Yet Evaluated | Not Yet Evaluated | | | - | - | - | Under Secondary Rev |

詳細はここをクリック

Showing 1 to 8 of 8 rows

Haploinsufficiency (HI) Score Details

HI Score: **3**

HI Evidence Strength: **Sufficient Evidence for Haploinsufficiency** (Disclaimer)

HI Disease: DiGeorge syndrome [Monarch](#)

HI Evidence: [PUBMED: 20301696](#)

McDonald-McGinn, Emanuel and Zackai. GeneReviews: 22q11.2 Deletion Syndrome.

[PUBMED: 27189754](#)

McDonald-McGinn et al. (2015) reviewed clinical findings in 22q11.2 deletion syndrome. See also PMID 21200182.

HI Evidence Comments:

Deletion of the 22q11.2 proximal (A-D) region is associated with DiGeorge/Velocardiofacial (DGS/VCFS) syndrome. Clinical findings are variable, but typically include congenital heart disease (particularly conotruncal malformations), palatal abnormalities (particularly velopharyngeal incompetence, cleft palate and bifid uvula), characteristic facial features, DD/ID, behavior problems, immune deficiency, and hypocalcemia. Most 22q11.2 (DGS/VCFS) deletions (>90 percent) are de novo. This deletion is enriched in the clinical population.

Additional relevant literature is summarized below:

Case-control studies:

PMID 25217958:

Coe et al. (2014): In a large-scale case-control comparison study of the relative prevalence of copy number variants in children with ID/DD, MCA, and other developmental phenotypes compared to controls, deletion of the recurrent 22q11.2 (DGS/VCFS) A-B region, the presumed critical region between LCR22A and -D, was observed in 158/29,085 cases versus 0/19,584 controls ($p=3.97E-36$; LR: Inf, CI: 43.9 to Inf).

GRCh37 Search Results

Location: chr22:18912231-21465672

Genes: On

Regions: Off

108
Total
Genes

8
Total
Regions

RegionをクリックしてoffにするとGenesのみの表記となる

Advanced Filters: None






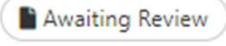












Search in table

GRCh37

Enter cytoband or genomic coordinates

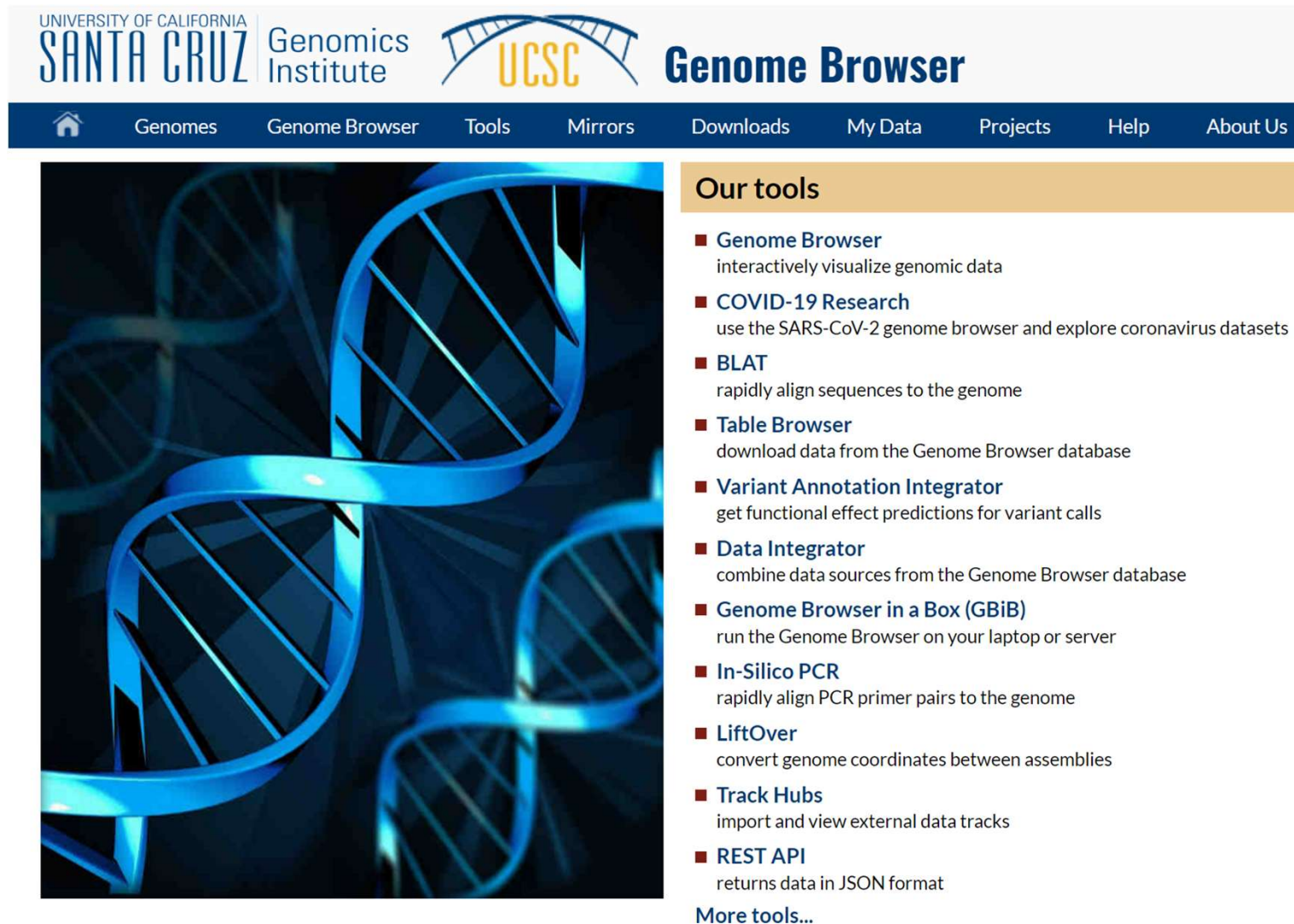
Go!

Showing 1 to 25 of 108 rows 25 rows per page

| Gene/Region | GRCh37 | HI Score | TS Score | OMIM | Morbid | %HI | pLI | LOEUF | Report |
|---|----------------------------|-------------------|-------------------|------|--------|-------|-----|-------|---|
|   PRODH | 22 18900287 18924066 | Not Yet Evaluated | Not Yet Evaluated | ✓ | ✓ | 45.38 | 0 | 1.1 |  |
|   DGCR5 | 22 18958011 19018755 | Not Yet Evaluated | Not Yet Evaluated | ✓ | | - | - | - |  |
|   LOC100287576 | 22 18985531 18987264 | -1 (Pseudogene) | -1 (Pseudogene) | | | - | - | - |  |
|   LOC100506454 | 22 19001864 19018742 | -1 (Pseudogene) | -1 (Pseudogene) | | | - | - | - |  |
|   DGCR5 | 22 19005347 19007761 | Not Yet Evaluated | Not Yet Evaluated | ✓ | | - | - | - |  |
|   DGCR5 | 22 19010137 19011063 | Not Yet Evaluated | Not Yet Evaluated | ✓ | | - | - | - |  |

UCSC Genome Browser

University of California
SANTA CRUZ



UNIVERSITY OF CALIFORNIA
SANTA CRUZ Genomics Institute UCSC Genome Browser

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Our tools

- **Genome Browser**
interactively visualize genomic data
- **COVID-19 Research**
use the SARS-CoV-2 genome browser and explore coronavirus datasets
- **BLAT**
rapidly align sequences to the genome
- **Table Browser**
download data from the Genome Browser database
- **Variant Annotation Integrator**
get functional effect predictions for variant calls
- **Data Integrator**
combine data sources from the Genome Browser database
- **Genome Browser in a Box (GBiB)**
run the Genome Browser on your laptop or server
- **In-Silico PCR**
rapidly align PCR primer pairs to the genome
- **LiftOver**
convert genome coordinates between assemblies
- **Track Hubs**
import and view external data tracks
- **REST API**
returns data in JSON format

[More tools...](#)

<https://http://www.genome.ucsc.edu/> (2022.5.7アクセス)

UCSC Genome Browser使用の流れ

1. Custom TracksにCNVデータを入力

CNV領域が表示される

- ✓ Zoom Outして領域全体を見渡しやすくする
- ✓ HighlightでCNV領域を見やすくする

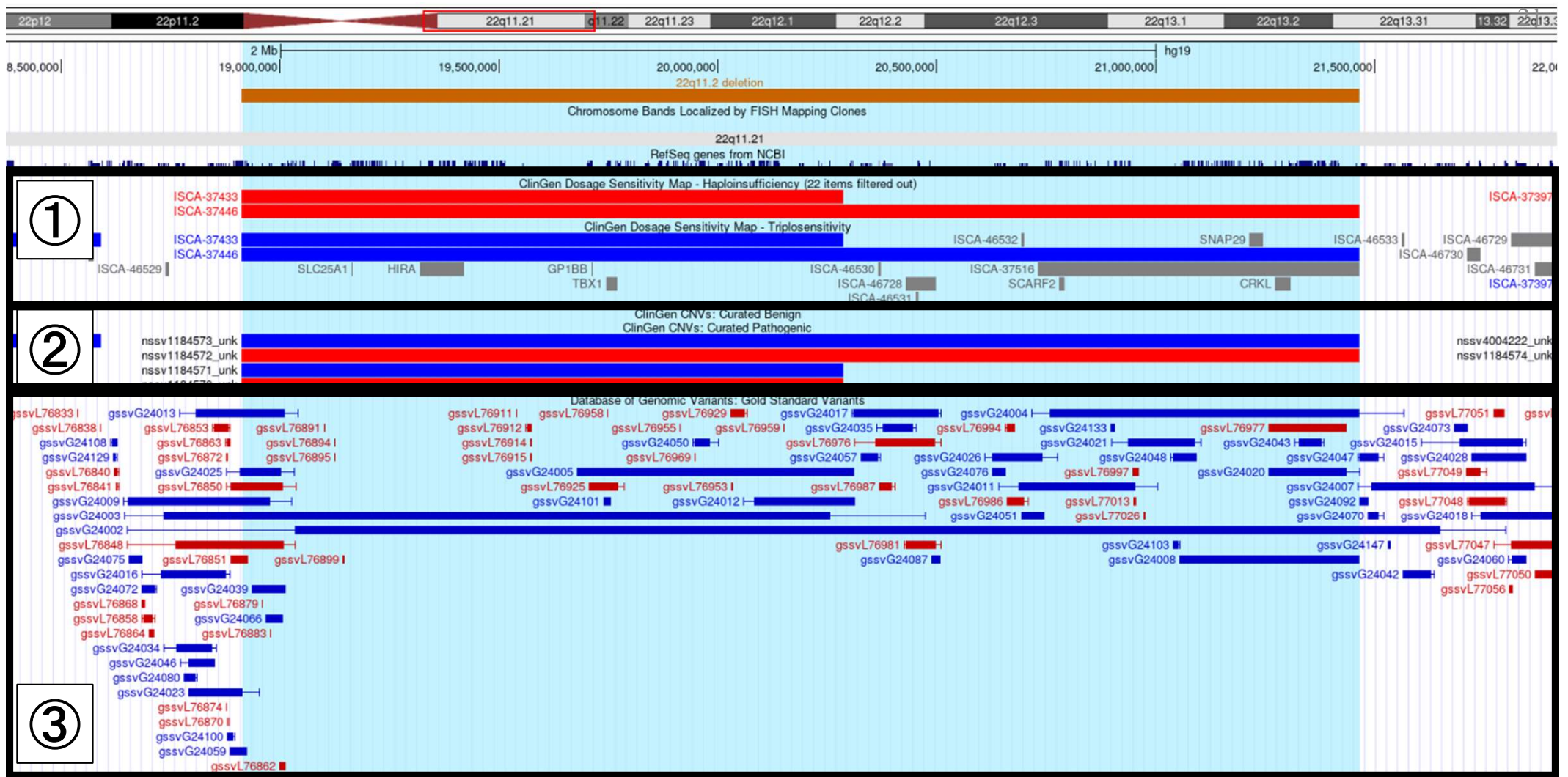
2. 必要な情報表示の選択をする

CNVの病原性評価に有用な表示情報(一例)

- ✓ Base position, Chromosome Band
- ✓ ClinGen (HI/TS Score)
- ✓ ClinGen CNVs (Curated Pathogenic/Benign)
- ✓ DGV Gold Standard (一般集団CNV→”Benign示唆”)

3. 表示内容から病原性を判断する

※詳細はAdvanced編 “データベース「UCSC」使用方法”を参照



① Dosage Sensitivityに関連した情報: ClinGen

② Curated Pathogenic/Benignの情報: ClinGen CNVs

③ 一般集団CNVデータベースの情報: DGV Gold Standard

利点

欠点(留意点)

DECIPHER

- HI, TSの根拠がわかりやすい
 - 数値化されたデータ
(HI/TS Score, pLI, LOEUF, %HI)



病原性判断がスムーズ



ClinGen Dosage Sensitivity

- HI, TSの根拠がわかりやすい
 - 数値化されたデータ
(HI/TS Score, pLI, LOEUF, %HI)
- GRCh37が選択可能

UCSC Genome Browser

- 視覚的にCNVを表示できる
(CNV)
- DECIPHERやClinGenで生じる
表示特性による誤診が防ぎやすい
- HI, TS関連情報のほか、一般集団データベースの情報も表示可能
(たくさんの情報を統合表示できる)

- GRCh38を基準としている
 - CMA結果はGRCh37準拠
 - データ入力時に”grch37”のが必須
- 入力したCNVを一部ないしすべて含むすべてのCNV関連情報が表示される
(表示内容の中から取捨選択が必要)
- 視覚的にCNV範囲が分かりにくい
- 入力したCNVを一部ないしすべて含むすべてのCNV関連情報が表示される
(表示内容の中から取捨選択が必要)
- 視覚的にCNV範囲が分かりにくい
- 表示したいデータの選択(操作)が煩雑
- 視覚表示に慣れるまでに時間がかかる
(通常の表示ではScore表示がない)

追記

ClinGen, DECIPHER, UCSCでは確立されたHI/TS領域の
病原性評価が可能

ただし、

「病原性が確立されていないCNVの検出」もありうる



「類似症例の文献検索」「報告症例との対比」

CMA結果解釈の留意点

- コピー数低下 (Loss)なのか増加 (Gain)なのかを間違えない
- 対応する症候群 (疾患) がハプロ不全 (欠失)、重複感受性どちらで生じるのかも必ず確認する

Loss, Gainの間違いは誤診に直結！

- データベースはそれぞれ特性あり。最初は「使いやすい」「使い慣れた」からの使用でOK
- 病原性が確立されたCNV以外の評価には文献検索が必要