Supplemental Material 3: Case Examples

The following case examples are meant to illustrate how one would use the CNV scoring metrics to assess a given CNV. This supplement includes the following examples:

- Example Case 1: A multi-gene deletion illustrating use of case-level evidence. This example also illustrates how classification may be affected by additional information regarding patient phenotype.
- Example Case 2: A multi-gene duplication with final classification of Uncertain. This example provides insight on how to prioritize genes within the CNV for evaluation.
- Example Case 3: An inherited deletion with final classification of Benign. This example illustrates use of population data.
- Example Case 4: A recurrent duplication with final classification of Pathogenic. This example illustrates use of the ClinGen Dosage Sensitivity Map.

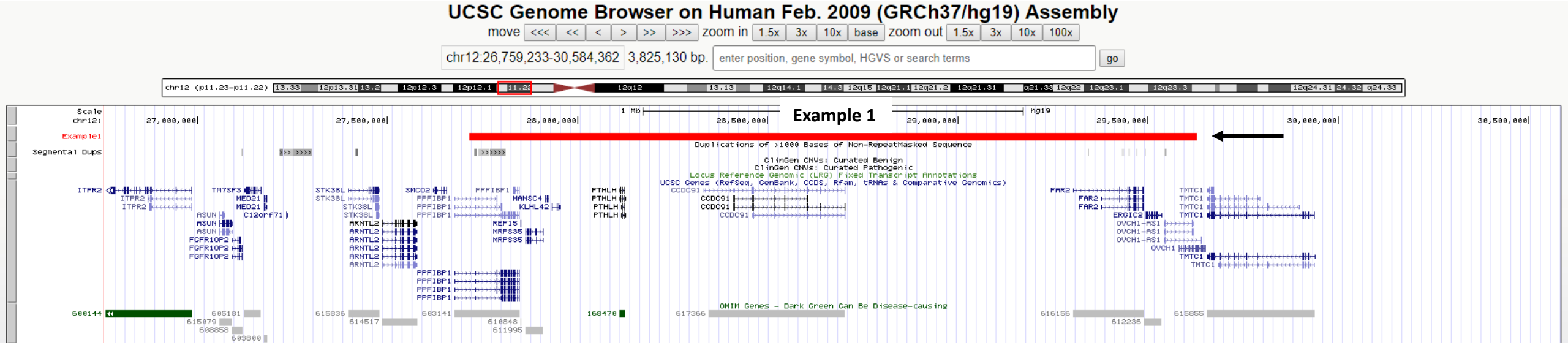
These classifications were made based on evidence available as of August 2019. Classifications may change over time as additional evidence emerges. Additional case examples will be provided at www.clinicalgenome.org.

Example Case 1: arr(GRCh37) 12p11.23p11.22 (2771551_29628080)x1

Case 1: Deletion with case-level evidence

- arr(GRCh37)12p11.23p11.22(27715516_29628080)x1 dn
- No additional clinical information provided
- Use the LOSS scoring metric

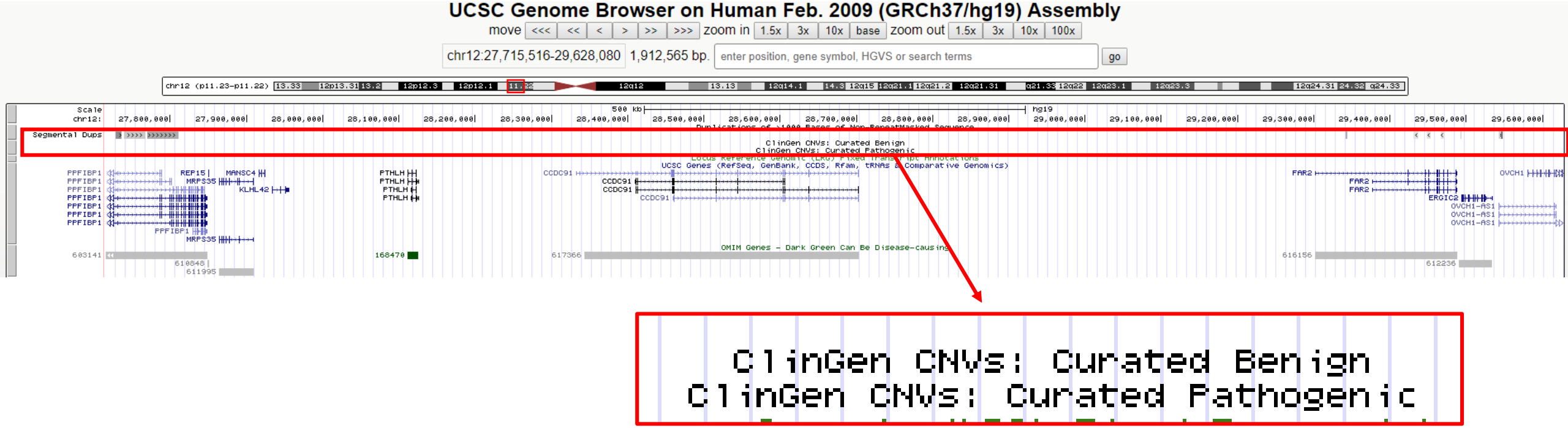
Section 1: Initial Assessment of Genomic Content



- Would apply category 1A (contains protein-coding or other known functionally important elements), as this deletion includes several protein-coding genes
- 0 points awarded, continue evaluation

Total: 0 points

Section 2: Overlap with Established/Predicted HI or Established Benign Genes/Genomic Regions



There are no established HI or benign genes/genomic regions within the observed interval.

Section 2: Overlap with Established/Predicted HI or Established Benign Genes/Genomic Regions

ClinGen Dosage Sensitivity Curation Page

Location Search Results

https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/

Submitted location: chr12:27,715,516-29,628,080

"N/A" indicates that this gene has not yet been evaluated

Location search results

Gene Symbol	Haploinsufficiency score	Triplosensitivity score	Curation Status	Region Location (GRCh37)	ExAC pLI	OMIM	Relationship to Submitted Location	ISCA ID
PTHLH	2	1	Complete	chr12:28,111,017-28,124,916	0.921	168470	Contained	ISCA-17209
PPFIBP1	N/A	N/A	Awaiting Review	chr12:27,677,045-27,848,497	0.0	603141	Overlap	ISCA-10221
LOC100506578	N/A	N/A	Reopened	chr12:27,745,391-27,746,972			Contained	ISCA-16181
REP15	N/A	N/A	Awaiting Review	chr12:27,849,428-27,850,566	0.001	610848	Contained	ISCA-30108
HMGB1P49	N/A	N/A	Awaiting Review	chr12:27,855,271-27,855,839			Contained	ISCA-32863
MRPS35	N/A	N/A	Awaiting Review	chr12:27,863,706-27,909,237	0.041	611995	Contained	ISCA-7936
MANSC4	N/A	N/A	Awaiting Review	chr12:27,915,599-27,924,209			Contained	ISCA-8474
KLHL42	N/A	N/A	Awaiting Review	chr12:27,933,187-27,955,973	0.439		Contained	ISCA-2790
RN7SKP15	N/A	N/A	Awaiting Review	chr12:27,959,015-27,959,323			Contained	ISCA-38098

One gene has been evaluated by the ClinGen Dosage Sensitivity group, but HI score is 2.

Section 2: Overlap with Established/Predicted HI or Established Benign Genes/Genomic Regions

- Sections 2A-2G: N/A
  - Does not overlap an established HI gene or genomic region
  - Does not overlap an established benign gene or genomic region

Total: 0 points

Section 2H: Haploinsufficiency Predictors

- Use DECIPHER to quickly assess pLI score (currently pulled from ExAC) and HI Index

Search results for 'position:12:27715516-29628080' (Refine Search)

Open-access patients 55 | CNV Syndromes 0 | DDD Research Variants 2 | Genes 14

Results | Browser

Genes: 1 to 10 of 10 (out of 14 total)

Show: OMIM Morbid DDG2P Protein coding Filter...

Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
PTHLH	12 28111017 28125638	parathyroid hormone like hormone	✔	✔	Y	5.77	0.92	View
FAR2	12 29302036 29493913	fatty acyl-CoA reductase 2	✔	-	-	65.22	0.92	View
KLHL42	12 27932953 27955973	kelch like family member 42	-	-	-	53.28	0.44	View
ERGIC2	12 29490285 29534122	ERGIC and golgi 2	✔	-	-	12.72	0.32	View
MRPS35	12 27863706 27909228	mitochondrial ribosomal protein S35	✔	-	-	39.99	0.04	View
PPFIBP1	12 27676364 27848497	PPFIA binding protein 1	✔	-	-	48.49	0.00	View
REP15	12 27849428 27850566	RAB15 effector protein	✔	-	-	78.52	0.00	View
CCDC91	12 28286182 28732883	coiled-coil domain containing 91	✔	-	-	29.75	0.00	View
OVCH1	12 29565407 29650619	ovochymase 1	-	-	-	90.08	0.00	View
MANSC4	12 27915671 27924209	MANSC domain containing 4	-	-	-	83.29	-	View

DECIPHER GRCh37 About Browse DDD(UK) Search DECIPHER Join Login

Search results for 'position:12:27715516-29628080' (Refine Search)

Open-access patients 55 | CNV Syndromes 0 | DDD Research Variants 2 | Genes 14

Results | Browser

Genes: 1 to 10 of 10 (out of 14 total)

Show: OMIM Morbid DDG2P Protein coding Filter...

Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
PTHLH	12 28111017 28125638	parathyroid hormone like hormone	✔	✔	Y	5.77	0.92	View
FAR2	12 29302036 29493913	fatty acyl-CoA reductase 2	✔	-	-	65.22	0.92	View
KLHL42	12 27932953 27955973	kelch like family member 42	-	-	-	53.28	0.44	View
ERGIC2	12 29490285 29534122	ERGIC and golgi 2	✔	-	-	12.72	0.32	View
MRPS35	12 27863706 27909228	mitochondrial ribosomal protein S35	✔	-	-	39.99	0.04	View
PPFIBP1	12 27676364 27848497	PPFIA binding protein 1	✔	-	-	48.49	0.00	View
REP15	12 27849428 27850566	RAB15 effector protein	✔	-	-	78.52	0.00	View
CCDC91	12 28286182 28732883	coiled-coil domain containing 91	✔	-	-	29.75	0.00	View
OVCH1	12 29565407 29650619	ovochymase 1	-	-	-	90.08	0.00	View
MANSC4	12 27915671 27924209	MANSC domain containing 4	-	-	-	83.29	-	View

This gene has pLI of >0.90 AND HI <10% and looks promising! However, since we know the pLI is being pulled from ExAC, let's double check in gnomAD…

However…

gnomAD browser | Search by gene, region, or variant | About | Downloads | Terms | Contact

This is a new version of the gnomAD browser. The old version is available at http://gnomad-old.broadinstitute.org

PTHLH parathyroid hormone-like hormone

Dataset: gnomAD v2.1.1 | gnomAD SVs

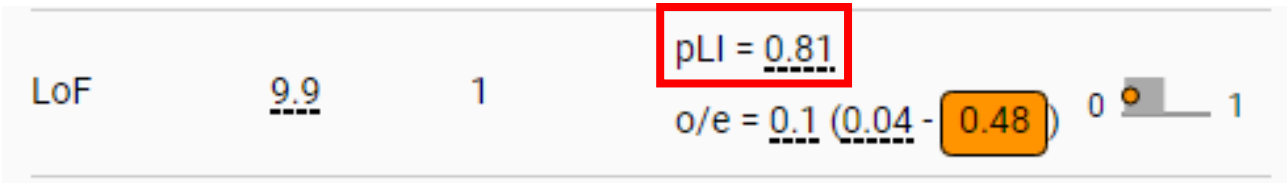
Ensembl gene ID	ENSG00000087494
Ensembl transcript ID	ENST00000395872 (canonical)
UCSC Browser	12:28111018-28125639
GeneCards	PTHLH
OMIM	168470

Gene Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	44.9	33	Z = 1.39 o/e = 0.74 (0.56 - 0.98)
Missense	107.7	74	Z = 1.15 o/e = 0.69 (0.57 - 0.83)
LoF	9.9	1	pLI = 0.81 o/e = 0.1 (0.04 - 0.48)

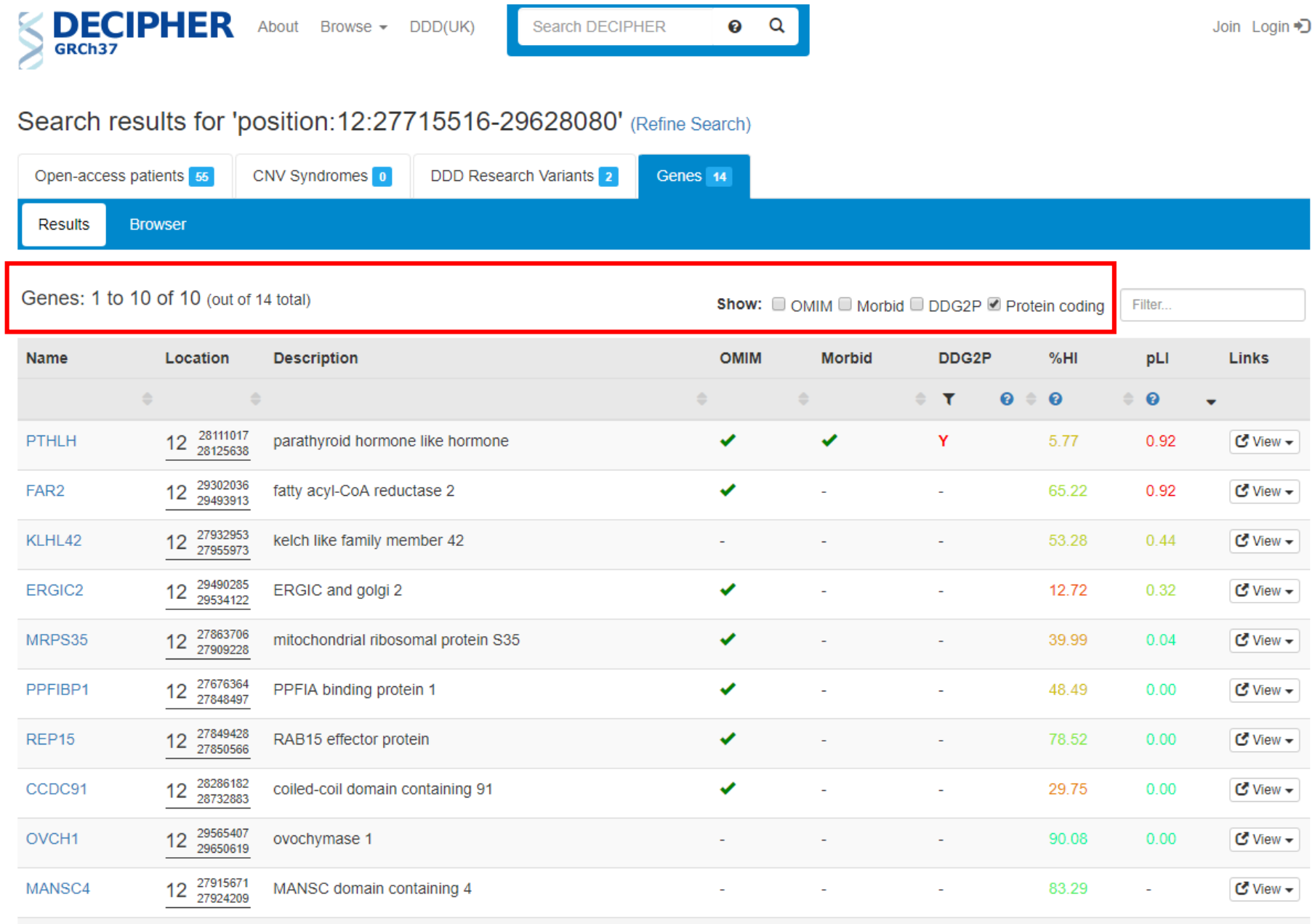
genome | Save plot

The pLI is no longer >0.90, AND the upper bound of the observed/expected confidence interval is >0.35. Do not assign points in category 2H.



Total: 0 points

Section 3: Evaluation of Gene Number



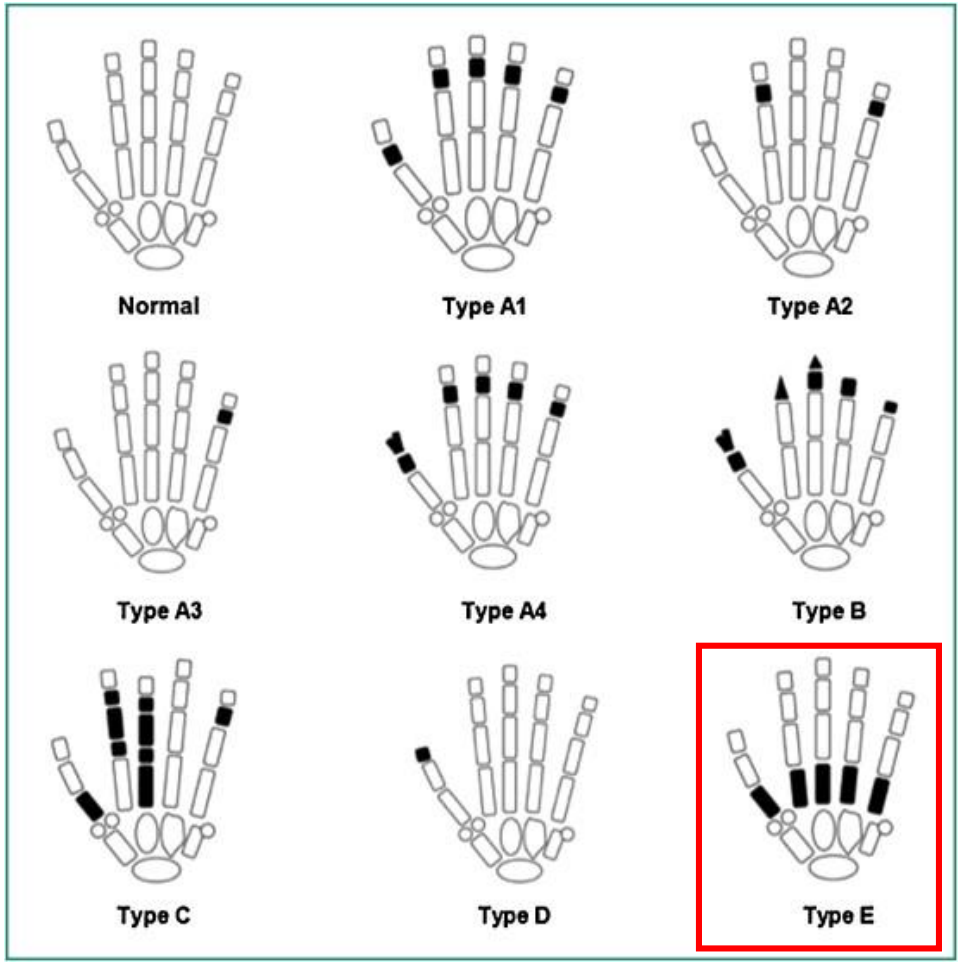
Total: 0 points

Section 4: Detailed Evaluation of Genomic Content

- Where to start the evaluation?
  - 10 protein-coding genes in the interval
  - Are any OMIM-Morbid?
    - Yes – *PTHLH*
      - Per OMIM: Associated with autosomal dominant Brachydactyly, type E2 (BDE)
      - Of note, this gene is the only gene in the interval with complementary DECIPHER HI and ExAC pLI scores
        - Though the pLI score is not within range when calculated using the gnomAD dataset, this still flags the gene as one of potential interest to follow-up on
  - If NO OMIM-Morbid genes:
    - Look at any genes documented in OMIM
    - Look at genes with complementary HI predictor scores
  - At any time: search the literature for the entire CNV interval
    - Keep in mind that results may be too broad for use (e.g., “12p11 deletions”)

PTHLH and Autosomal Dominant Brachydactyly Type E (BDE)

- Shortening of the digits, mainly in the metacarpals (III-V) and metatarsals
  - Wide variability in the number of digits affected, including within the same family
- May also be associated with short stature, delayed tooth eruption and/or oligodontia
- Intelligence generally unaffected



David et al. (2015). *Diagnostic and Interventional Imaging* 96(5):443-448

PTHLH and Autosomal Dominant Brachydactyly Type E (BDE)

- Brachydactyly *in general* may occur as an isolated malformation or as part of a syndrome
  - Searching OMIM clinical synopses for “brachydactyly” yields over 250 results
- Brachydactyly type E specifically is more rare
  - Similar OMIM search using “brachydactyly type E” yields 4 results
- Isolated BDE has also been associated with variants in *HOXD13*
  - Similar radiologic features are also seen in:
    - pseudopseudohypoparathyroidism (variants in *PPHP*) - this syndrome is also marked by cognitive defects and cataracts
    - Hypertension and brachydactyly syndrome (variants in *PDE3A*) – this syndrome is also marked by severe hypertension
    - 2q37 deletions (including *HDAC4*) – also marked by intellectual disability
- Given the relatively limited heterogeneity of BDE, this may be considered a “highly specific, but not necessarily unique” phenotype
  - Brachydactyly, type unspecified, would be considered “not highly specific and/or with high genetic heterogeneity”

De Novo variants

Category 4B→ Default: confirmed parental relationships = 0.30; assumed = 0.15; Range: 0-0.45

- Thomas-Teinturier et al. 2016 (PMID: 26640227):
  - The authors describe a female proband with BDE and no reported family history.
  - Single gene sequencing of PTHLH was performed, and a heterozygous de novo deletion was detected (c.101+3delAAGT).
  - Per the authors, "this alteration deletes four nucleotides from position 3–6 of intron V...[it] is predicted to cause a moderate decrease in the consensus sequence value of the natural donor splice site (WT 84.38; Mut 60.23; Var.% 28.62). As a result, novel additional potential donor sites with a higher consensus value could be created. Use of these sites is predicted to lead to aberrant transcripts with a premature stop codon, as in the first family."
  - Parental relationships were not confirmed.
  - Default for assumed parental relationships: 0.15
    - Opting to downgrade to 0.10: Other genetic causes of BDE were not effectively ruled out.

Total: 0.10 points

De Novo variants

Category 4B→ Default: confirmed parental relationships = 0.30; assumed = 0.15; Range: 0-0.45

- Pereda et al. 2017 (PMID: 28211986):
  - Female proband with BDE and no reported family history.
  - Single gene sequencing of PTHLH revealed a heterozygous c.166C>T (p.R56*) variant; this variant has been described in an unrelated individual with BDE by Jamsheer et al. 2016.
  - The variant was also detected in a mosaic state in the girl's unaffected father (10-20% of blood cells, not detected via buccal swab or hair sample).
    - Per ClinGen gene curation scoring guidelines, if a variant is detected in a mosaic state in the proband's parent, it can be "counted" as a *de novo* variant.
    - Maternal relationship was not confirmed.
  - Default for assumed parental relationships: 0.15
    - Opting to downgrade to 0.10: Other genetic causes of BDE were not effectively ruled out.

Total: 0.20 points

De Novo variants

Category 4B→ Default: confirmed parental relationships = 0.30; assumed = 0.15; Range: 0-0.45

- Jamsheer et al. 2016 (PMID: 26763883):
  - Female proband with BDE and no reported family history.
  - Single gene sequencing of *PTHLH* revealed a heterozygous c.166C>T (p.R56*) variant
    - This variant has been described in an unrelated individual with BDE by Pereda et al. 2017.
  - Variant was said to be *de novo*, but no information regarding confirmation of parental relationships was presented.
  - Default for assumed parental relationships: 0.15
    - Opting to downgrade to 0.10: Other genetic causes of BDE were not effectively ruled out.

Total: 0.30 points

Variants of unknown inheritance

Category 4E → Default: 0.10; Range: 0-0.15

- Klopocki et al. 2010 (PMID: 20170896):
  - One individual with a heterozygous nonsense variant (p.K120X) (Individual II-1 in Family 5)
  - The authors note that this variant is located more than 50bp away from the 3' boundary of the second-to-last exon, thus expected to undergo NMD.
  - The individual presented with brachydactyly type E (shortened metacarpals III-V), short stature, and oligodontia.
  - The individual was said to have an affected sister and nephew, though it is unclear whether these individuals were tested for the variant.
  - Parental testing was not performed.
  - Default points awarded (0.10).
  - Note: this category is being used because BDE is a relatively specific phenotype. If the phenotype under evaluation was non-specific, this category would not be used.

Total: 0.40 points

Segregation Among Similarly Affected Family Members

- Note: Segregations may be added across families.
- Reyes et al. 2018 (PMID: 30458061):
  - The authors describe a female proband with brachydactyly type E (BDE) and no evidence for abnormal calcium or phosphate regulation.
  - Whole exome sequencing (WES) revealed "a novel heterozygous A>G change at nucleotide -3 up-stream of PTHLH exon 3 that encodes the last two amino acids of the pro-sequence and the mature PTHrP...[resulting in] a heterozygous insertion of genomic nucleotides -2 and -1 causing a frame-shift after residue 34 of the preprosequence and thus 29 novel residues without homology to PTHrP or any other protein."
  - This variant was also found in her affected mother, maternal aunt, and monozygous twin sons, but not in her unaffected daughter or sister.
  - Number of segregations counted = 3

Total # of Segregations: 3

Segregation Among Similarly Affected Family Members

- Bae et al. 2018 (PMID:29947179):
  - Male proband with BDE and normal calcium and phosphate levels.
  - WES of the proband, his affected mother, and his unaffected father revealed a shared c.169C>T (p.Arg57*) variant for the proband and his affected mother.
  - The family history indicates that there are at least 7 other affected relatives across 4 generations of the family, though no other individuals were tested.
  - Number of segregations counted = 1
    - Only counting genotype +/phenotype + individuals

Total # of Segregations: 4

Segregation Among Similarly Affected Family Members

- Thomas-Teinturier et al. 2016 (PMID: 26640227):
  - The authors describe a female proband and her mother with BDE.
  - Single gene sequencing of *PTHLH* was performed. A heterozygous deletion, c.47_101+73del128 "compris[ing] bases 47–101 of exon V and 73 bases of intron V (total deletion: 128 bp)" was found in the proband and her mother (NM_198965.1).
  - Per the authors: "The c.47_101þ73del128 defect removes the canonical donor site of exon V. Consequently, new potential splice sites localized before or after the deletion breakpoint are predicted to be used. These aberrant splicings are expected to produce transcripts with premature stop codon 5' to the last 50 nucleotides of the penultimate exon [Holbrook et al., 2004], which are predicted to be degraded by nonsense-mediated mRNA decay."
  - Number of segregations counted = 1

Total # of Segregations: 5

Segregation Among Similarly Affected Family Members

- Jamsheer et al. 2016 (PMID: 26763883):
  - The authors describe a female proband with BDE; her full sister and father were also reported to be affected.
  - Single-gene sequencing revealed a NM_198965.1 c.258delC (p.N87Tfs*18) in exon 4 of PTHLH in the proband and her affected family members, but not in her unaffected mother, sister, or paternal aunt.
  - Number of segregations counted = 2

- Total number of segregations across all families = 7
- Category 4H: 7 or more segregations = 0.45 points

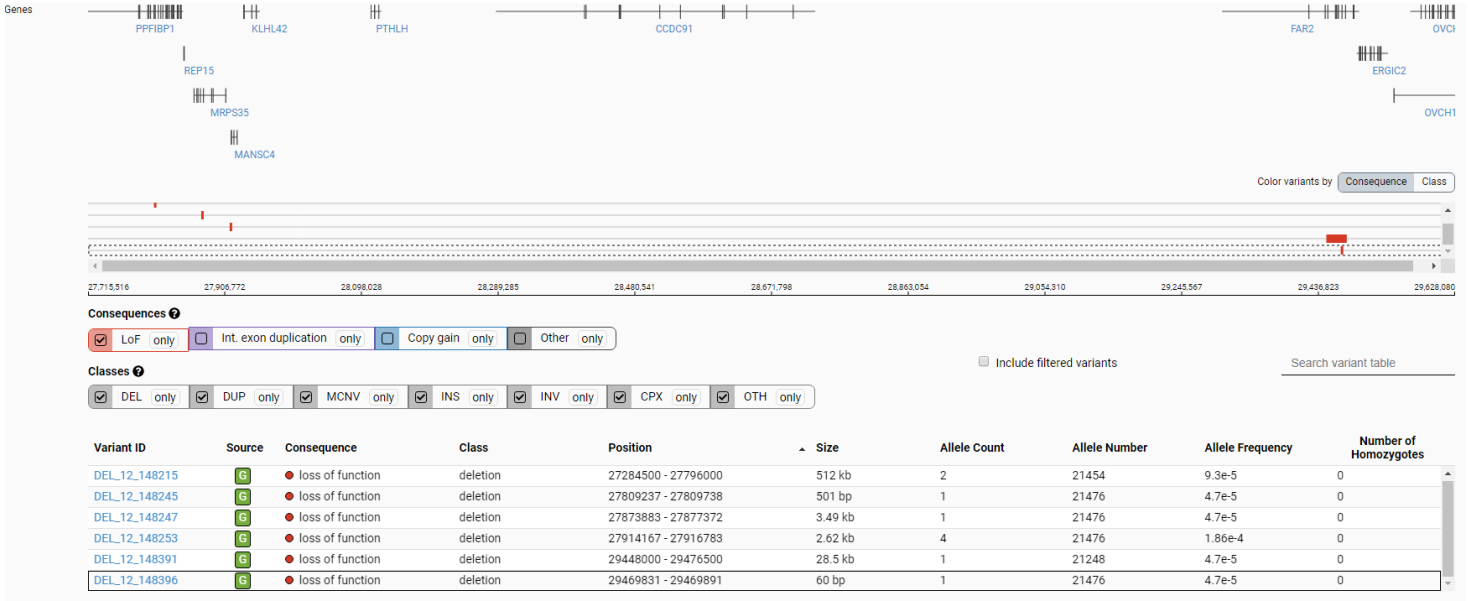
Total # of Segregations: 7

Total: 0.85 points

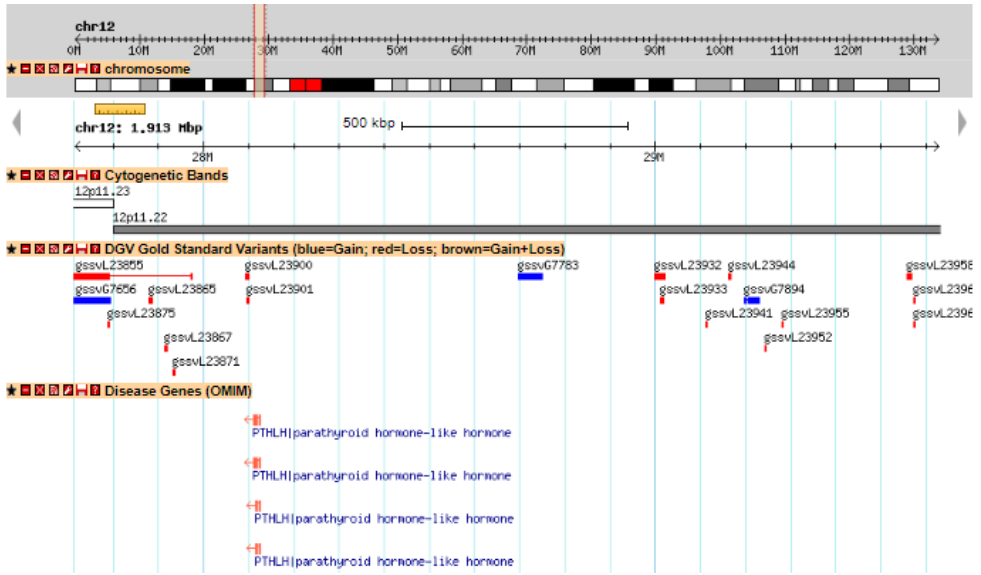
Case-control and population evidence

- No known case-control evidence available for the observed CNV (Categories 4L-4N = 0 points)
- Population Data:

gnomAD SVs



DGV Gold Standard Set



Multiple small events are observed throughout the region. Though some in DGV are at high frequencies, they are much smaller than the observed CNV, and the information is not enough to constitute “anti” evidence for this CNV (0 points).

Total: 0.85 points

Section 5: Evaluation of Inheritance Pattern/Family History for Patient Being Studied

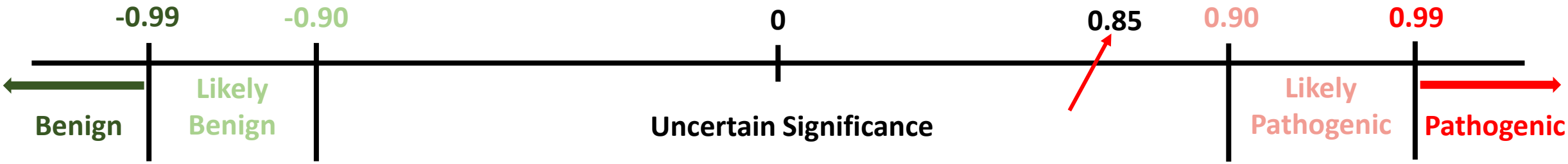
- Though we know this variant is *de novo*, we have not been provided any information about the patient's phenotype or family history.
  - Without any further information, the most appropriate category would be 5F, "Inheritance information is unavailable or uninformative" (0 points).
- Option: Call the ordering provider and request additional information.
  - If the proband is noted to have brachydactyly: Could award an additional 0.15-0.30 points (depending on whether parental relationships are confirmed) for another *de novo* observation of a highly specific (but not necessarily unique) phenotype (Category 5A).
    - This additional information would bring the total points to 1.0 (or greater) for a classification of PATHOGENIC.

What if my patient does not have BDE?

- The full body of documented supporting evidence should not be completely discounted because your patient does not have BDE.
  - Total points do not suddenly change from 0.85 to -0.90 or less; there is no cause to classify this CNV as “likely benign” or “benign” simply because your patient does not exhibit the expected phenotype.
- Carefully evaluate what this may mean in the context of the other evidence.
- Just as consistent phenotype information from your patient can be scored as a case, inconsistent phenotype information can also be scored as a case (Category 4D; Default = 0 points, Range = 0 to -0.30 points).
  - Decide whether this represents a lack of information or truly negative information.
  - For example: Who determined that the proband did not have brachydactyly?
    - Did the doctor’s office call the parent, ask if the child had short fingers, and the parent told them he didn’t? (Consider 0 points)
    - Did the child have an X-ray of the hand where BDE was specifically ruled out by a radiologist? (Consider negative points)
- Classifications should be driven by all available evidence, not swayed by a single case; this is the intent of the “uncoupling” recommendation.

What if no additional phenotypic information is available?

- Current point total without proband phenotype: 0.85 points



- Very close to likely pathogenic (LP) – is variant of uncertain significance really appropriate?
- Use your clinical judgement
  - Evaluate the full body of evidence – does it warrant an upgrade? Is there any contradictory information?
  - Document your rationale

In this case…

- There are additional pieces of information available about *PTHLH* that could support an argument to bump this CNV up to LP:
  - Deletions overlapping our example CNV (including *PTHLH*) have been observed segregating within families with BDE (Klopocki *et al*. 2010 [PMID: 20170896]; Huang *et al*. 2019 [PMID:31283647]).
    - Why don't we just count these in our evaluation? Both of these publications represent additional segregation information; we have already obtained the maximum segregation score of 0.45 points.
  - Missense variants in *PTHLH* with functional information suggesting a loss of function mechanism have also been observed in individuals with BDE (Klopocki et al. 2010 [PMID: 20170896]).

Conclusion

- Final points based on publicly available evidence: 0.85
- Classification may change as additional evidence becomes available:
  - If phenotype information confirms proband has BDE:
    - This is an additional case - add 0.15-0.30 points (depending on confirmation of parental relationships) (Category 5A [4B])
    - Classification: Pathogenic
  - If contradictory evidence is available (e.g., your patient is radiologically confirmed not to have BDE):
    - This additional (contradictory) case information could subtract up to 0.30 points from your total. (Category 5A [4D]). Final points could range anywhere from 0.85 to 0.55 points.
    - In any scenario, the total number of points remain in the "Uncertain" range.
  - If phenotype information is unavailable or uninformative (no contradictory evidence):
    - Additional, unscored evidence supports the upgrade from 0.85 points to 0.90 points (evidence documented on previous slide)
    - Classification: Likely Pathogenic

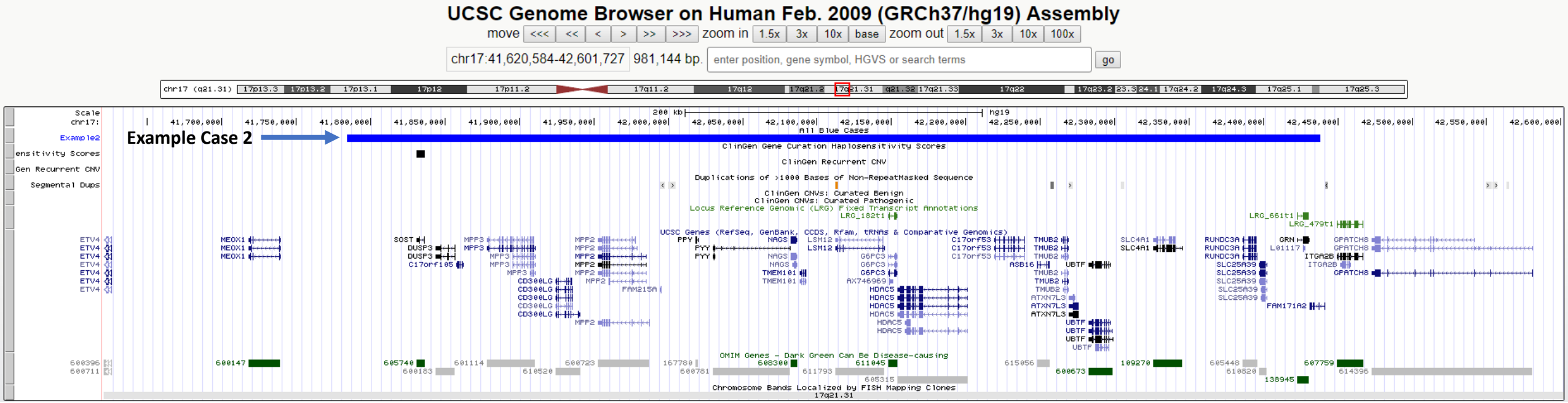
Example Case 2:

arr(GRCh37)
17q21.31(41784l0_42438203)x3

arr(GRCh37)17q21.31(41784108_42438203)x3

- No clinical information provided
- No parental testing available
- Use the GAIN scoring metric

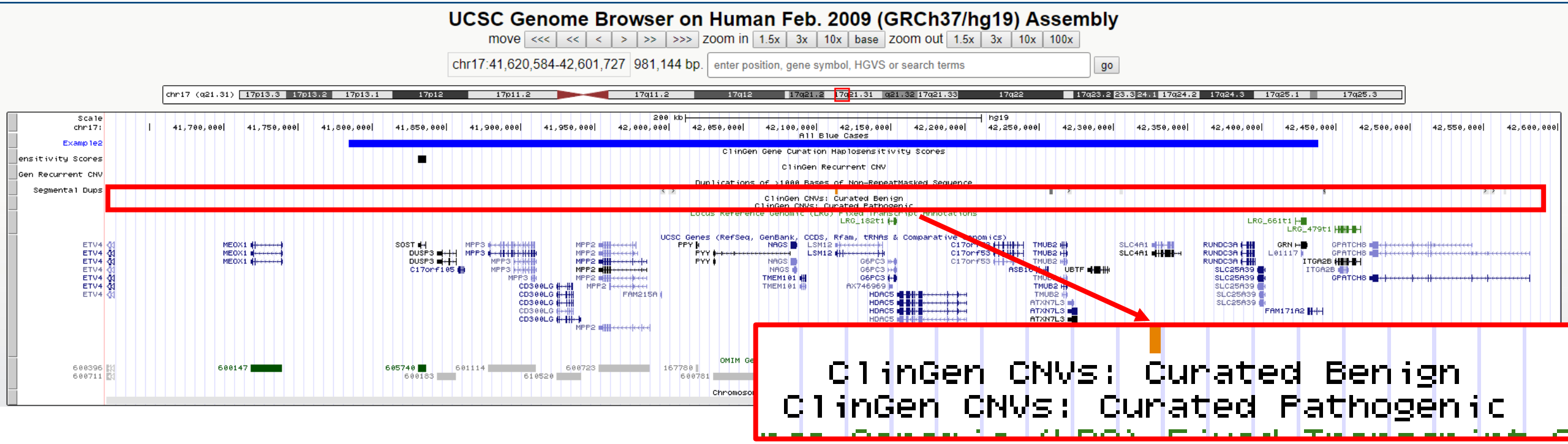
Section 1: Initial Assessment of Genomic Content



- Would apply category 1A ("contains protein-coding or other known functionally important elements"), as this duplication includes several protein-coding genes
- 0 points awarded, continue evaluation

Total: 0 points

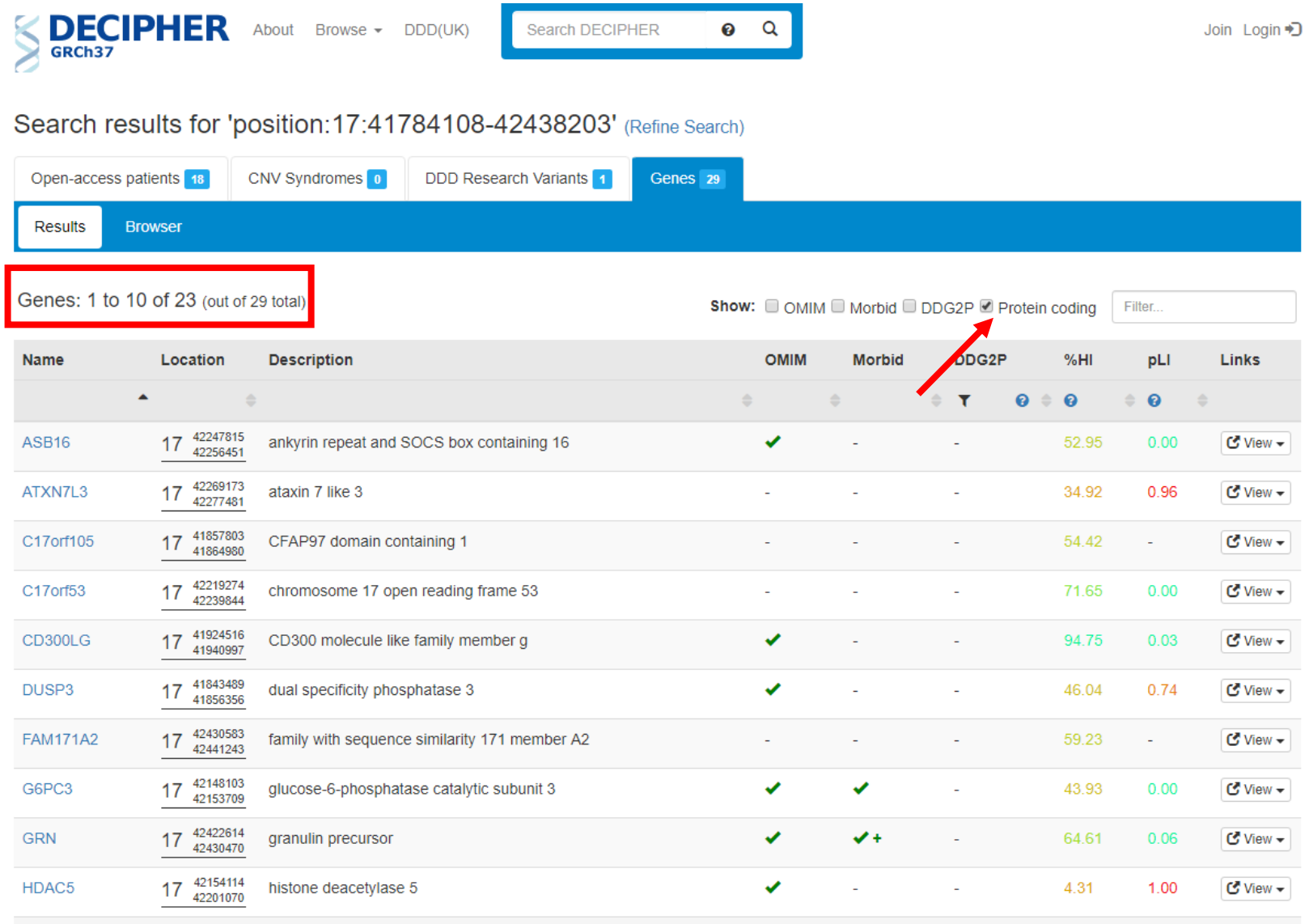
Section 2: Overlap with Established Triplosensitive (TS), Haploinsufficient (HI) or Benign Genes/Genomic Regions



- There are no established TS, HI, or Benign genes/genomic regions within the observed interval (0 points).

Total: 0 points

Section 3: Evaluation of Gene Number

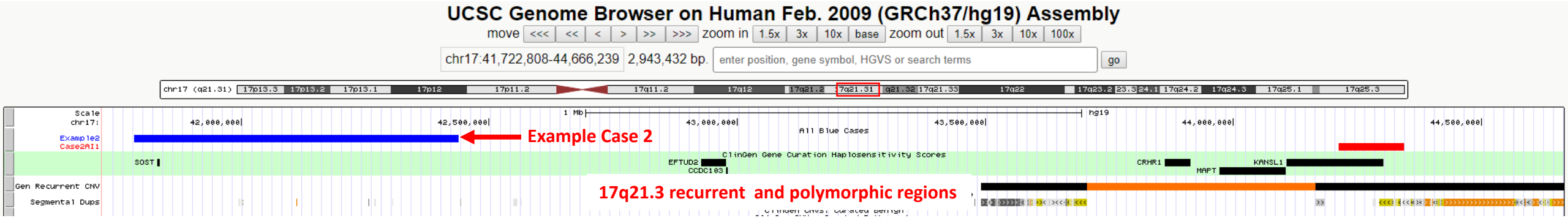


Section 3: Evaluation of Gene Number

- 23 protein-coding genes
  - Category 3A (“0-34 genes”) → 0 points
- DECIPHER is a useful resource for identifying which genes in the interval may be most appropriate to pursue within Section 4 (OMIM Morbid, OMIM, etc.)
  - List is sortable

Total: 0 points

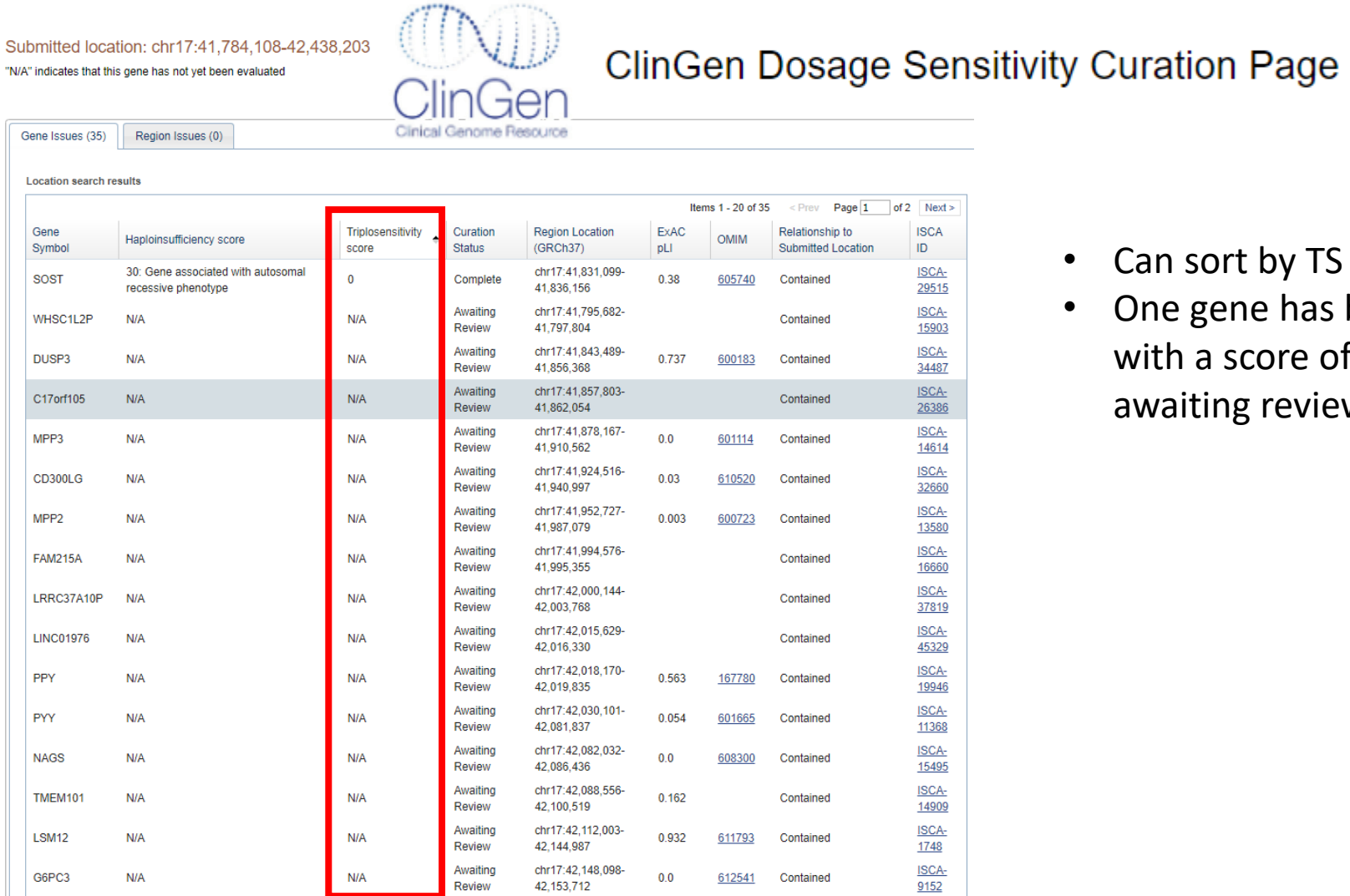
Section 4: Detailed Evaluation of Genomic Content Using Published Literature, Public Databases, and/or Internal Lab Data



- Starting literature search with “17q21.31” may produce irrelevant results due to other well-studied recurrent regions in this area.
- Consider searching for evidence related to genes within the interval.

Which genes should I start with?

- Has ClinGen evaluated any genes in this region?



Submitted location: chr17:41,784,108-42,438,203

"N/A" indicates that this gene has not yet been evaluated

ClinGen Clinical Genome Resource

ClinGen Dosage Sensitivity Curation Page

Gene Issues (35) | Region Issues (0)

Location search results

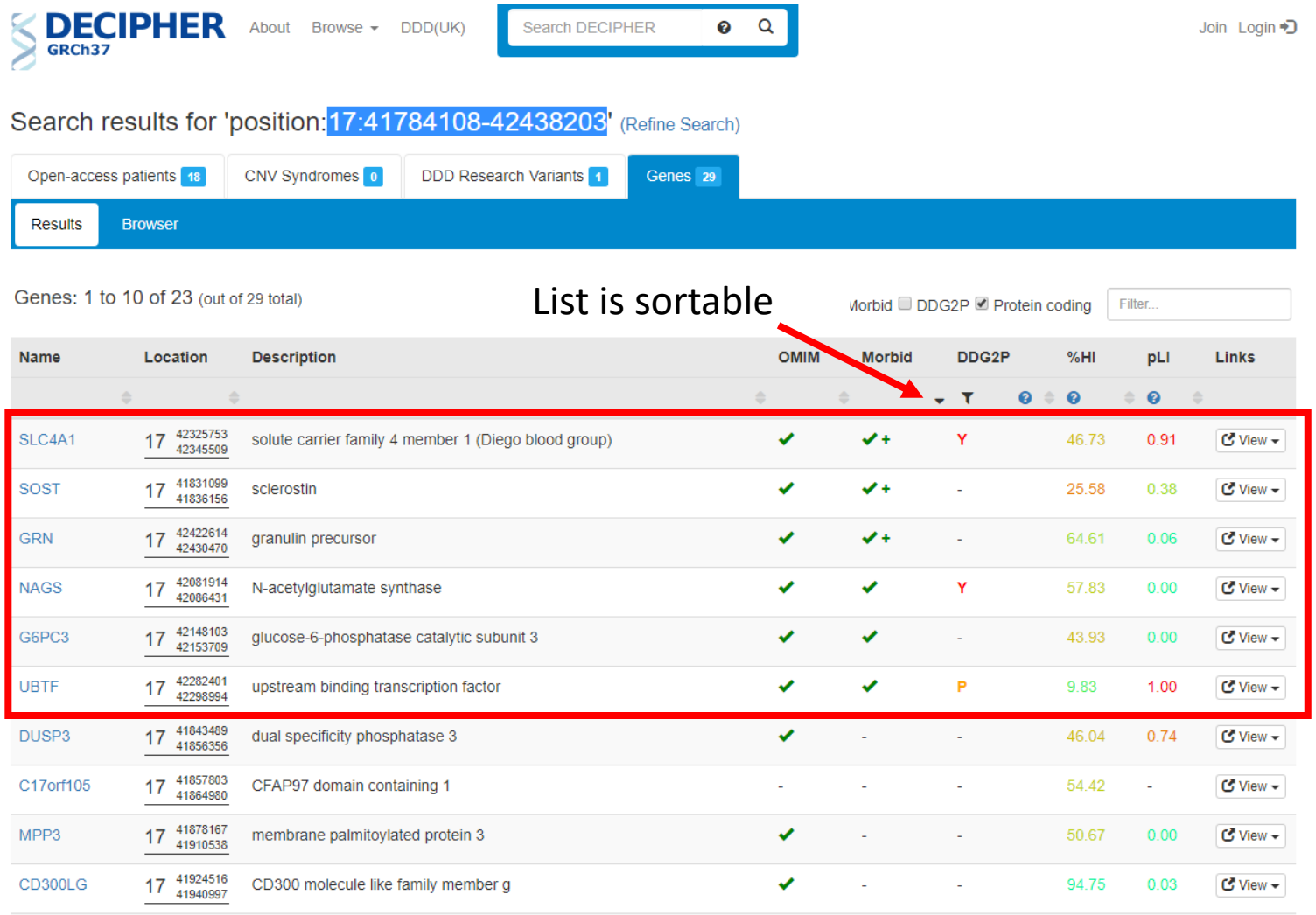
Items 1 - 20 of 35 < Prev Page 1 of 2 Next >

Gene Symbol	Haploinsufficiency score	Triplosensitivity score	Curation Status	Region Location (GRCh37)	ExAC pLI	OMIM	Relationship to Submitted Location	ISCA ID
SOST	30: Gene associated with autosomal recessive phenotype	0	Complete	chr17:41,831,099-41,836,156	0.38	605740	Contained	ISCA-29515
WHSC1L2P	N/A	N/A	Awaiting Review	chr17:41,795,682-41,797,804			Contained	ISCA-15903
DUSP3	N/A	N/A	Awaiting Review	chr17:41,843,489-41,856,368	0.737	600183	Contained	ISCA-34487
C17orf105	N/A	N/A	Awaiting Review	chr17:41,857,803-41,862,054			Contained	ISCA-26386
MPP3	N/A	N/A	Awaiting Review	chr17:41,878,167-41,910,562	0.0	601114	Contained	ISCA-14614
CD300LG	N/A	N/A	Awaiting Review	chr17:41,924,516-41,940,997	0.03	610520	Contained	ISCA-32660
MPP2	N/A	N/A	Awaiting Review	chr17:41,952,727-41,987,079	0.003	600723	Contained	ISCA-13580
FAM215A	N/A	N/A	Awaiting Review	chr17:41,994,576-41,995,355			Contained	ISCA-16660
LRRC37A10P	N/A	N/A	Awaiting Review	chr17:42,000,144-42,003,768			Contained	ISCA-37819
LINC01976	N/A	N/A	Awaiting Review	chr17:42,015,629-42,016,330			Contained	ISCA-45329
PPY	N/A	N/A	Awaiting Review	chr17:42,018,170-42,019,835	0.563	167780	Contained	ISCA-19946
PYY	N/A	N/A	Awaiting Review	chr17:42,030,101-42,081,837	0.054	601665	Contained	ISCA-11368
NAGS	N/A	N/A	Awaiting Review	chr17:42,082,032-42,086,436	0.0	608300	Contained	ISCA-15495
TMEM101	N/A	N/A	Awaiting Review	chr17:42,088,556-42,100,519	0.162		Contained	ISCA-14909
LSM12	N/A	N/A	Awaiting Review	chr17:42,112,003-42,144,987	0.932	611793	Contained	ISCA-1748
G6PC3	N/A	N/A	Awaiting Review	chr17:42,148,098-42,153,712	0.0	612541	Contained	ISCA-9152

- Can sort by TS score
- One gene has been evaluated with a score of 0; others are awaiting review.

Which genes should I start with?

- Are there any OMIM Morbid genes in the region?



- Yes! Next step: check the mechanism.

OMIM Morbid Genes in the Region

- All genes completely encompassed within duplicated interval
- Review of OMIM record and quick literature search indicates that triplosensitivity is not an established mechanism for any of these disorders
- Would not take any of these genes further through the scoring metric

- *SLC4A1*
  - Cryohydrocytosis (AD)
  - Ovalocytosis, SA type (AD)
  - Renal tubular acidosis, distal (AD)
  - Renal tubular acidosis, distal (AR)
  - Spherocytosis, type 4 (AD)
- *SOST* (ClinGen TS Score 0)
  - Craniodiaphyseal dysplasia (AD)
  - Sclerosteosis 1 (AR)
  - Van Buchem disease (AR)
- *GRN*
  - Aphasia, primary progressive (AD)
  - Ceroid lipofuscinosis, neuronal, 11 (AR)
  - Frontotemporal lobar degeneration with ubiquitin-positive inclusions (AD)
- *NAGS*
  - N-acetylglutamate synthase deficiency (AR)
- *G6PC3*
  - Dursun syndrome (AR)
  - Neutropenia, severe congenital 4 (AR)
- *UBTF*
  - Neurodegeneration, childhood-onset, with brain atrophy (AD)

Total: 0 points

What next?

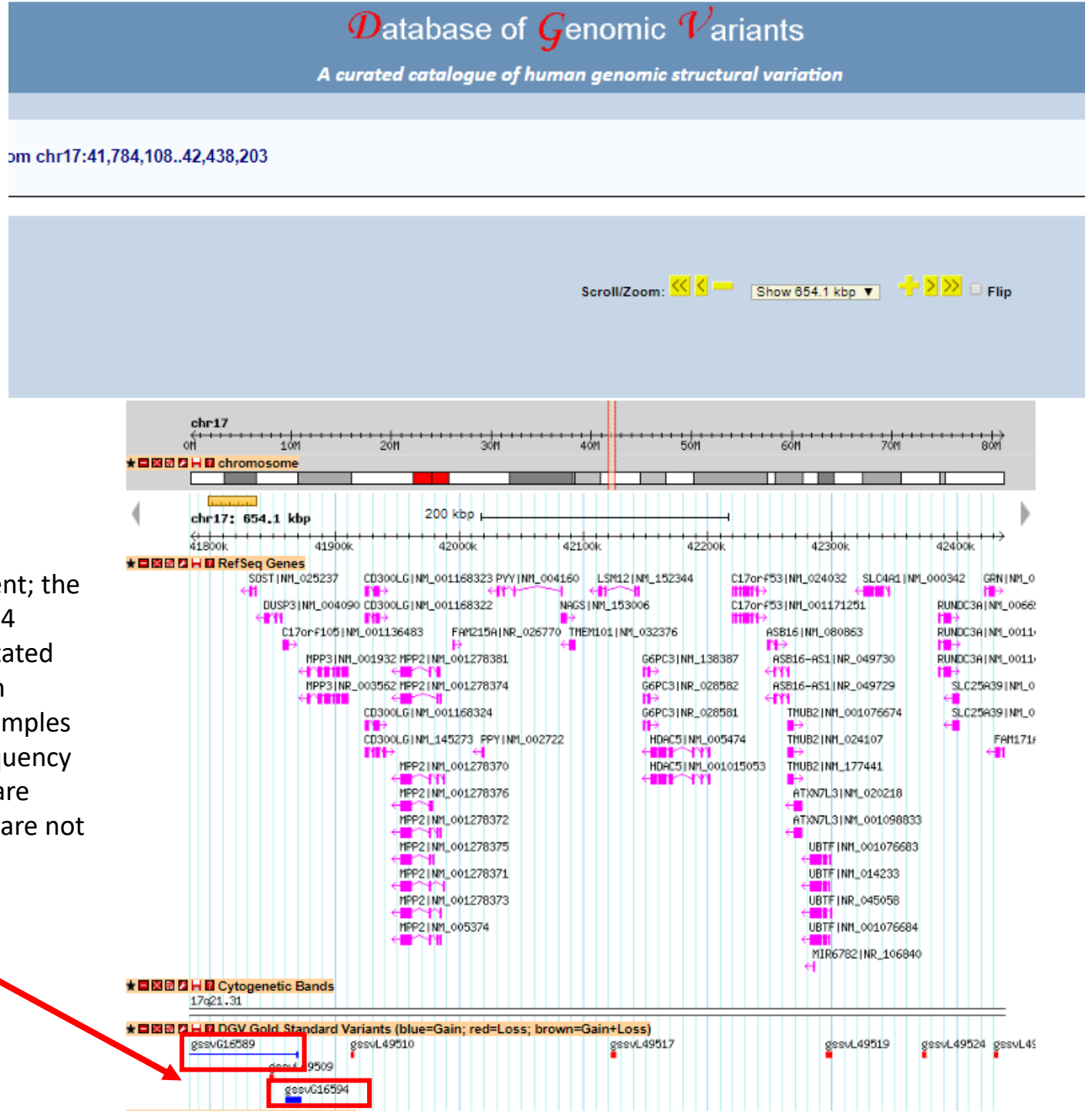
- Quick review of remaining OMIM genes – no evidence to suggest any of these genes causes disease by triplosensitivity (0 points)

Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
DUSP3	17 41843489 41856356	dual specificity phosphatase 3	✔	-	-	46.04	0.74	View
MPP3	17 41878167 41910538	membrane palmitoylated protein 3	✔	-	-	50.67	0.00	View
CD300LG	17 41924516 41940997	CD300 molecule like family member g	✔	-	-	94.75	0.03	View
MPP2	17 41952725 41987068	membrane palmitoylated protein 2	✔	-	-	53.42	0.00	View
PPY	17 42018172 42019836	pancreatic polypeptide	✔	-	-	56.98	0.56	View
PYY	17 42030106 42081837	peptide YY	✔	-	-	58.10	0.05	View
LSM12	17 42112003 42144987	LSM12 homolog	✔	-	-	8.10	0.93	View
HDAC5	17 42154114 42201070	histone deacetylase 5	✔	-	-	4.31	1.00	View
ASB16	17 42247815 42256451	ankyrin repeat and SOCS box containing 16	✔	-	-	52.95	0.00	View
RUNDC3A	17 42385781 42396039	RUN domain containing 3A	✔	-	-	46.63	0.96	View
SLC25A39	17 42396993 42402238	solute carrier family 25 member 39	✔	-	-	58.14	0.00	View

Section 4: Detailed Evaluation of Genomic Content

- Sections 4A-4K are non-applicable for this particular duplication
  - None of the included genes has evidence to suggest triplosensitivity as a disease mechanism
- Next: Evaluate for case-control and population data
  - This CNV is not recurrent; unlikely to be part of case-control studies
  - Check population data in gnomAD, DGV gold standard datsets

gnomAD – No relevant copy gains found



Two small duplications are present; the top duplication was observed in 4 samples in 2 studies and has a stated frequency of 0.03%. The bottom duplication was observed in 2 samples from 2 studies with a stated frequency of 0.08%. Both of these events are much smaller than our CNV and are not relevant to our case (0 points).

Section 5: Evaluation of Inheritance Patterns/Family History of Patient Being Studied

- No information was provided on the patient's presentation or their family history. Parental testing was not performed.
  - Category 5F: Inheritance information is unavailable or uninformative (0 points).
- Total points: 0 Proposed Classification: Uncertain
- Should this be Likely Benign (LB)?
  - In this scenario, there is no concrete evidence supporting pathogenicity, but also no concrete evidence supporting a classification of "benign."
  - While we cannot say definitively that this CNV has no effect on current or future phenotype, we can convey the sentiment that this is unlikely to cause a phenotype in the report (see Example Report 7).
- Should we test parents?
  - In this scenario, where our baseline score is 0, testing parents would not garner enough points to take this CNV out of the VUS range.

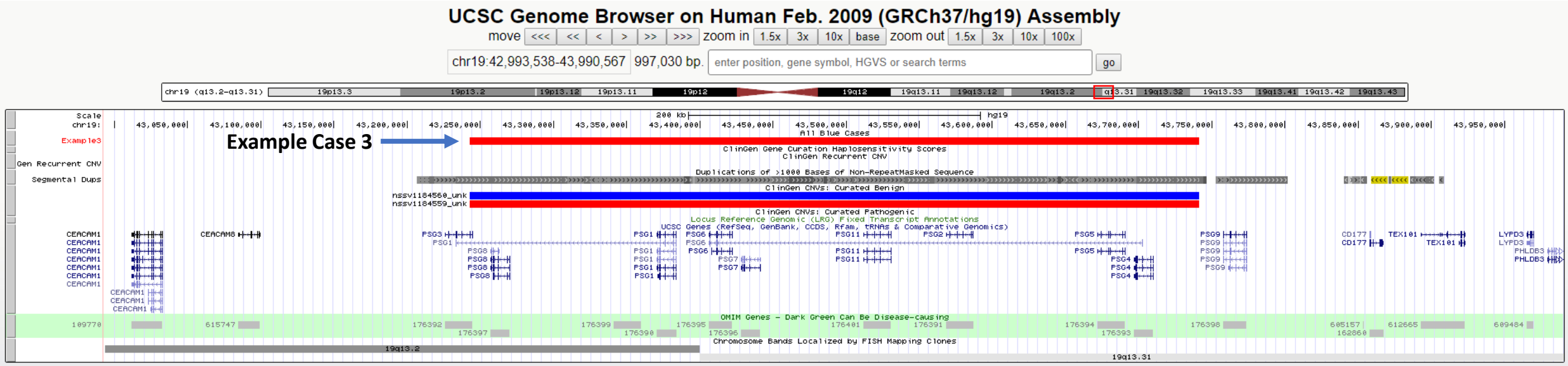
Example Case 3:

arr(GRCh37) 19q13.3 (43242796_43741310) x 1

arr(GRCh37) 19q13.3(43242796_43741310) x 1 mat

- Reason for referral: 3-year-old male referred for aniridia and genitourinary anomalies
- Inherited from apparently normal mother
- Use the LOSS scoring metric

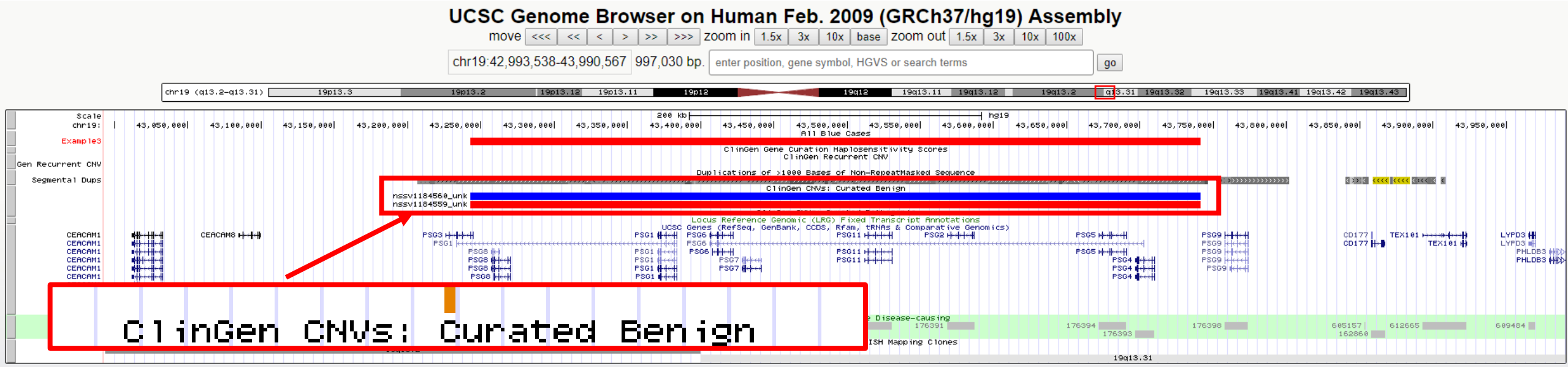
Section 1: Initial Assessment of Genomic Content



- Would apply category 1A ("contains protein-coding or other known functionally important elements"), as this duplication includes several protein-coding genes
- 0 points awarded, continue evaluation

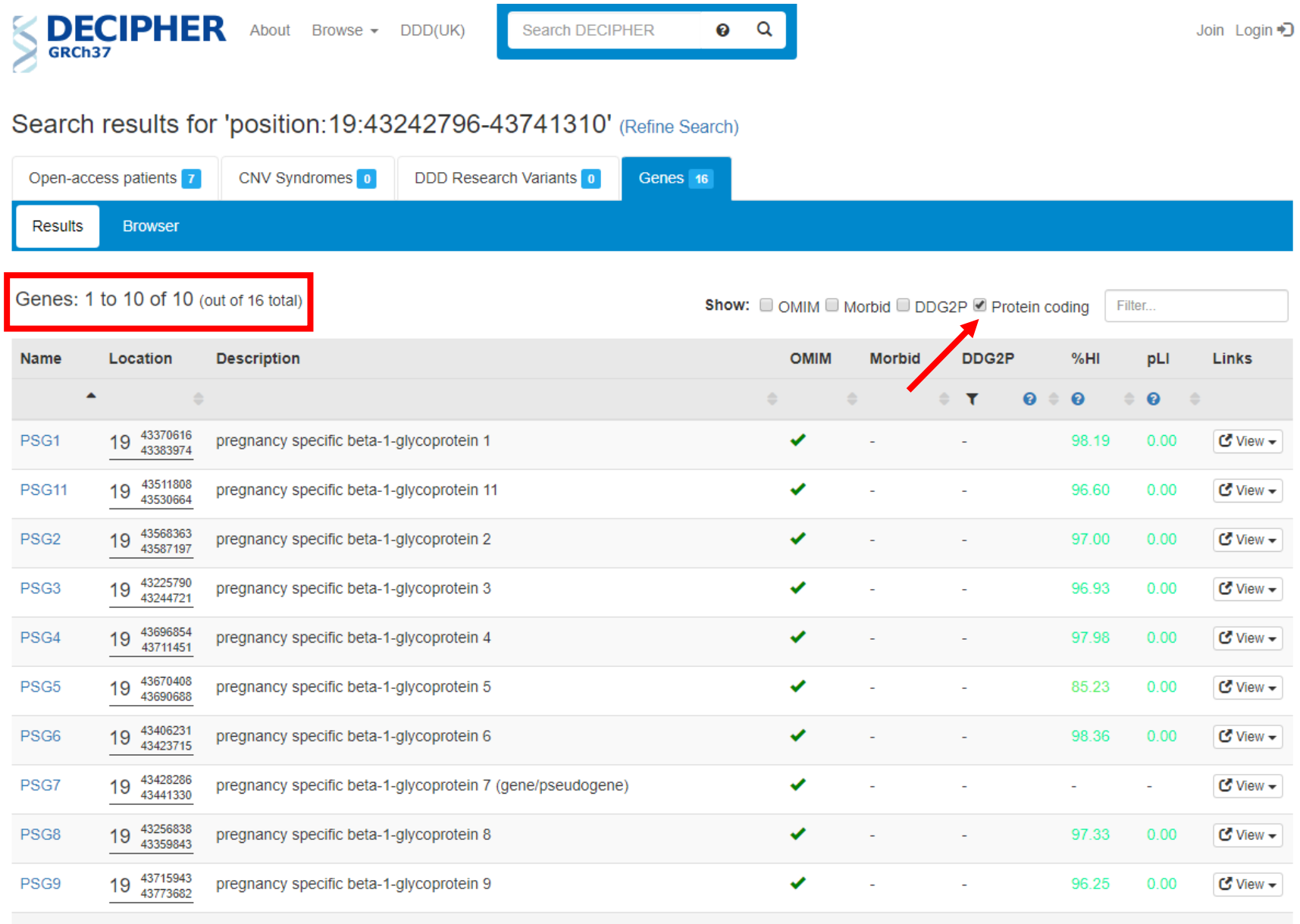
Total: 0 points

Section 2: Overlap with Established/Predicted Haploinsufficient (HI) or Established Benign Genes/Genomic Regions



- This deletion completely encompasses a ClinGen “dosage sensitivity unlikely” genomic region (category 2F).
- This warrants -1.0 points and a classification of BENIGN.
- However, for the sake of this example, we will continue the evaluation as if this region wasn’t already curated by ClinGen.

Section 3: Evaluation of Gene Number



Section 3: Evaluation of Gene Number

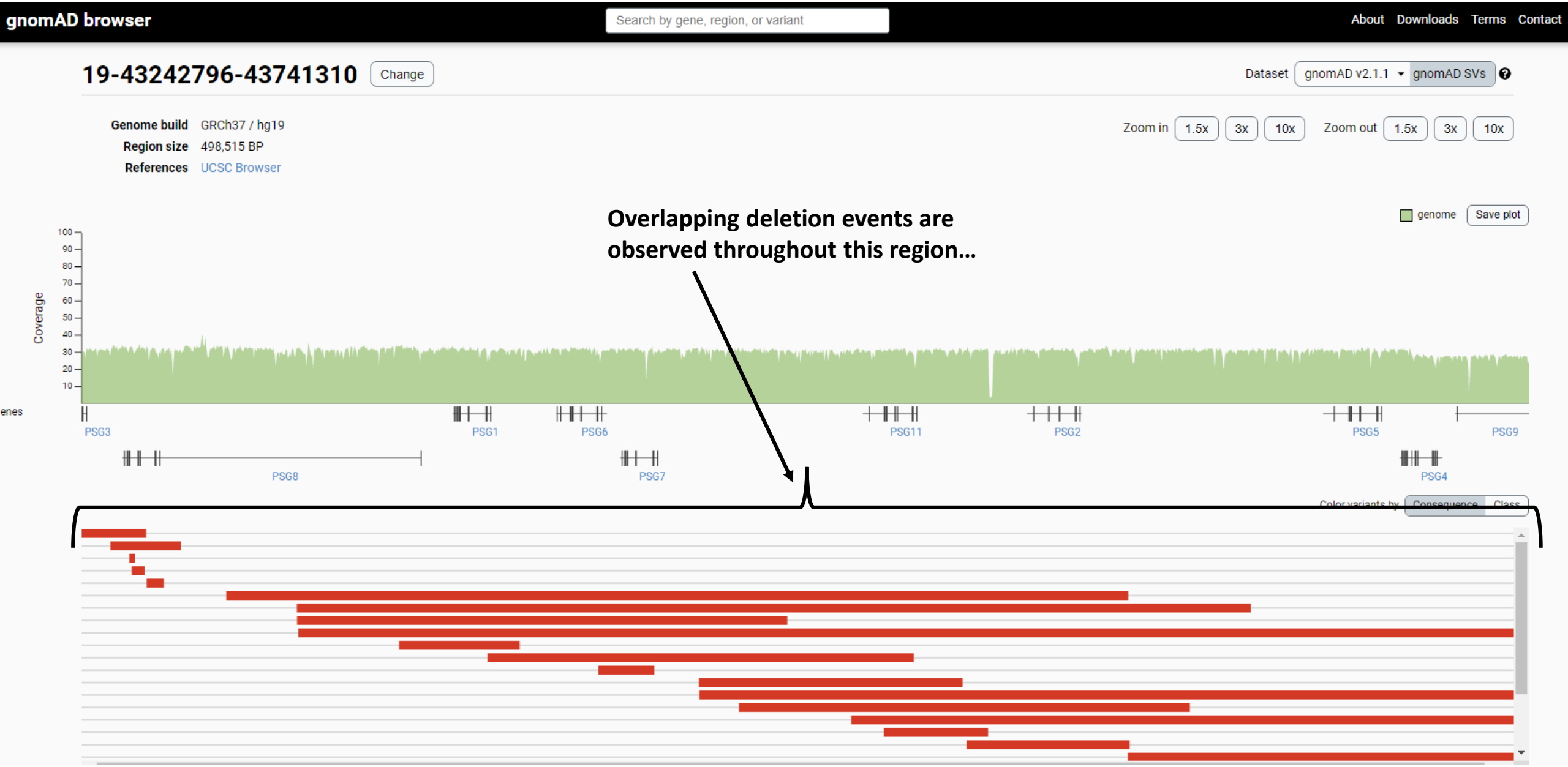
- 10 protein-coding genes
  - Category 3A (“0-24 genes”) → 0 points
  - Note: multiple members of the same gene family are within the CNV. If the genes within the gene family are not known to be associated with disease, consider counting them as 1 (as opposed to 10 distinct genes) to avoid artificially inflating the gene count. In this scenario, there are less than 24 genes any way, so this doesn’t affect the score.

Total: 0 points

Section 4: Detailed Evaluation of Genomic Content Using Published Literature, Public Databases, and/or Internal Lab Data

- Sections 4A-4K are non-applicable for this particular deletion
  - None of the included genes has evidence to suggest a relationship with human disease
- Next: Evaluate population data (Category 4O)
  - gnomAD, DGV gold standard datsets

Total: 0 points


gnomAD browser
Search by gene, region, or variant
About
Downloads
Terms
Contact
19-43242796-43741310
Change
Dataset
gnomAD v2.1.1
gnomAD SVs
Genome build GRCh37 / hg19
Region size 498,515 BP
References UCSC Browser
Zoom in
1.5x
3x
10x
Zoom out
1.5x
3x
10x
genome
Save plot
Overlapping deletion events are observed throughout this region…
Coverage
100
90
80
70
60
50
40
30
20
10
PSG3
PSG1
PSG6
PSG11
PSG2
PSG5
PSG9
PSG8
PSG7
PSG4
Color variants by
Consequence
Class

…at very high frequencies (some as high as almost 3.5%!)

43,242,796 43,292,647 43,342,498 43,392,350 43,442,201 43,492,053 43,541,904 43,591,755 43,641,607 43,691,458 43,741,310

Consequences

☑ pLoF only ☐ Int. exon duplication only ☐ Copy gain only ☐ Other only

Classes

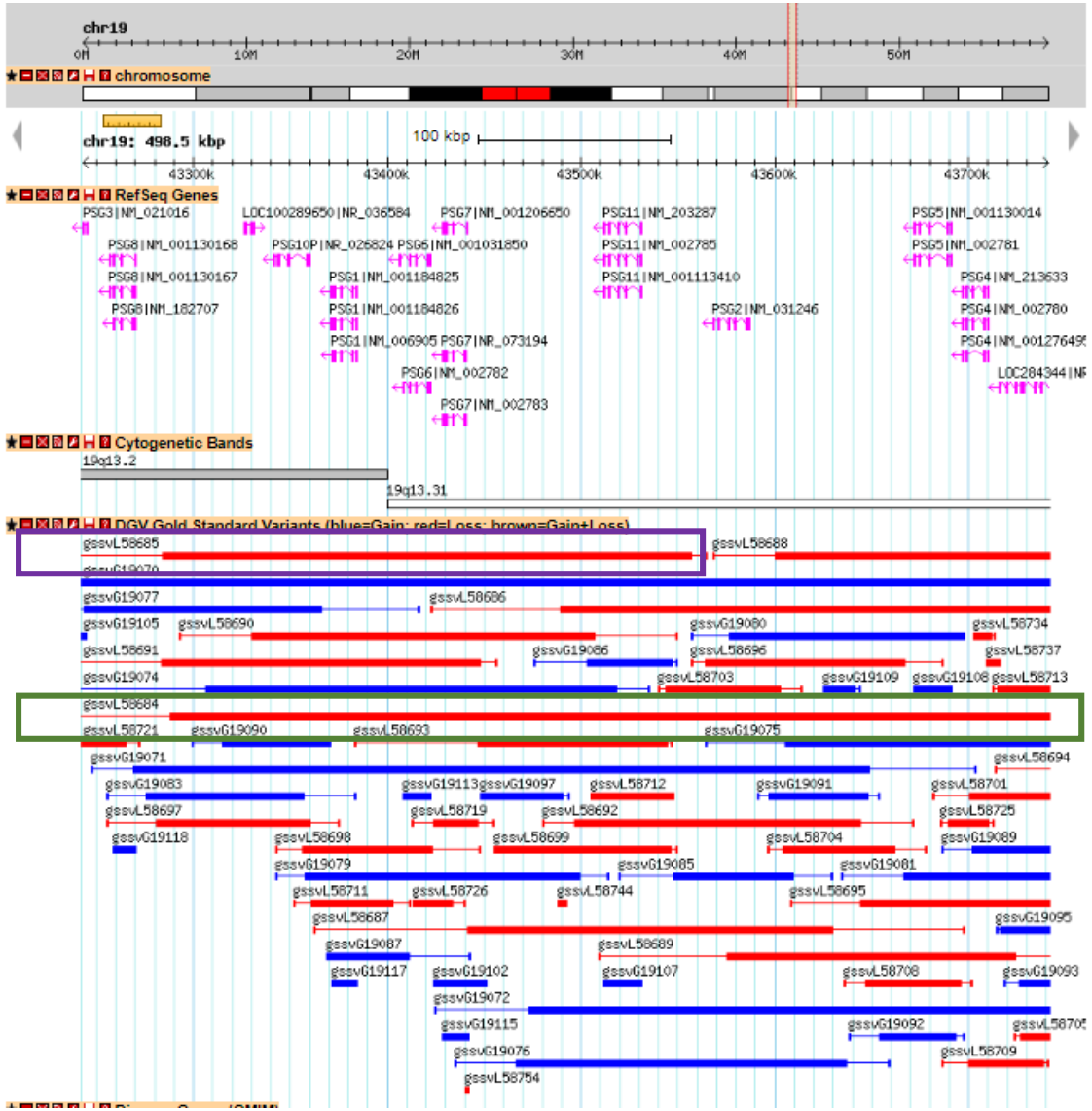
☑ DEL only ☑ DUP only ☑ MCNV only ☑ INS only ☑ INV only ☑ CPX only ☑ OTH only

☐ Include filtered variants

Search variant table

Variant ID	Source	Consequence	Class	Position	Size	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
DEL_19_203513	G	loss of function	deletion	43240000 - 43265000	25.0 kb	14	21156	6.62e-4	1
DEL_19_203516	G	loss of function	deletion	43252700 - 43277025	24.3 kb	67	20884	3.21e-3	1
DEL_19_203517	G	loss of function	deletion	43259179 - 43261136	1.96 kb	2	21476	9.3e-5	0
DEL_19_203519	G	loss of function	deletion	43260063 - 43264479	4.42 kb	1	21476	4.7e-5	0
DEL_19_203520	G	loss of function	deletion	43265181 - 43271102	5.92 kb	68	21280	3.19e-3	1
DEL_19_203524	G	loss of function	deletion	43292603 - 43603303	311 kb	538	21476	2.51e-2	6
DEL_19_203526	G	loss of function	deletion	43316928 - 43645531	329 kb	538	21476	2.51e-2	6
DEL_19_203527	G	loss of function	deletion	43316984 - 43485863	169 kb	538	21476	2.51e-2	6
DEL_19_203528	G	loss of function	deletion	43317433 - 43745515	428 kb	208	21436	9.7e-3	7
DEL_19_203533	G	loss of function	deletion	43352131 - 43393674	41.5 kb	510	21476	2.37e-2	6
DEL_19_203535	G	loss of function	deletion	43382581 - 43529434	147 kb	539	21476	2.51e-2	6
DEL_19_203539	G	loss of function	deletion	43420778 - 43440033	19.3 kb	569	21462	2.65e-2	10
DEL_19_203542	G	loss of function	deletion	43455418 - 43546255	90.8 kb	492	21360	2.3e-2	5
DEL_19_203543	G	loss of function	deletion	43455566 - 43789432	334 kb	676	21468	3.15e-2	9
DEL_19_203544	G	loss of function	deletion	43469163 - 43624537	155 kb	454	21122	2.15e-2	10
DEL_19_203550	G	loss of function	deletion	43507886 - 43753337	245 kb	676	21468	3.15e-2	9
DEL_19_203553	G	loss of function	deletion	43519150 - 43555000	35.9 kb	747	21472	3.48e-2	12
DEL_19_203555	G	loss of function	deletion	43547647 - 43603780	56.1 kb	519	21448	2.42e-2	8
DEL_19_203560	G	loss of function	deletion	43603132 - 43790133	187 kb	679	21474	3.16e-2	9
DEL_19_203563	G	loss of function	deletion	43645614 - 43745072	99.5 kb	491	21216	2.31e-2	8

Similar information in DGV…



Multiple high-frequency deletions across the region; highest frequency (purple box) is 2.88%; event encompassing most of our CNV (green box) is at 1.39%.

Total: -1 points

Section 5: Evaluation of Inheritance Patterns/Family History of Patient Being Studied

- Inherited from apparently normal mother
  - Should we continue to deduct points, using category 5B?
  - You could, but in this case, this is not necessary, as we have already reached a classification of Benign.
  - CAUTION
    - What if the mother had the same phenotype as the patient? Would we add points using Category 5D, taking this variant out of “Benign”?
    - No!
      - Strong population data supports a Benign classification for this variant.
      - Just because the mother and patient share a phenotype does not make this the causative variant.
      - More likely: the mother and patient have a different, yet-to-be-identified variant causing the phenotype.
      - Always use clinical judgement.

- Total points: -1 Proposed Classification: Benign

Example Case 4:

arr[GRCh37]
22q11.21(18912231_21465672)x3

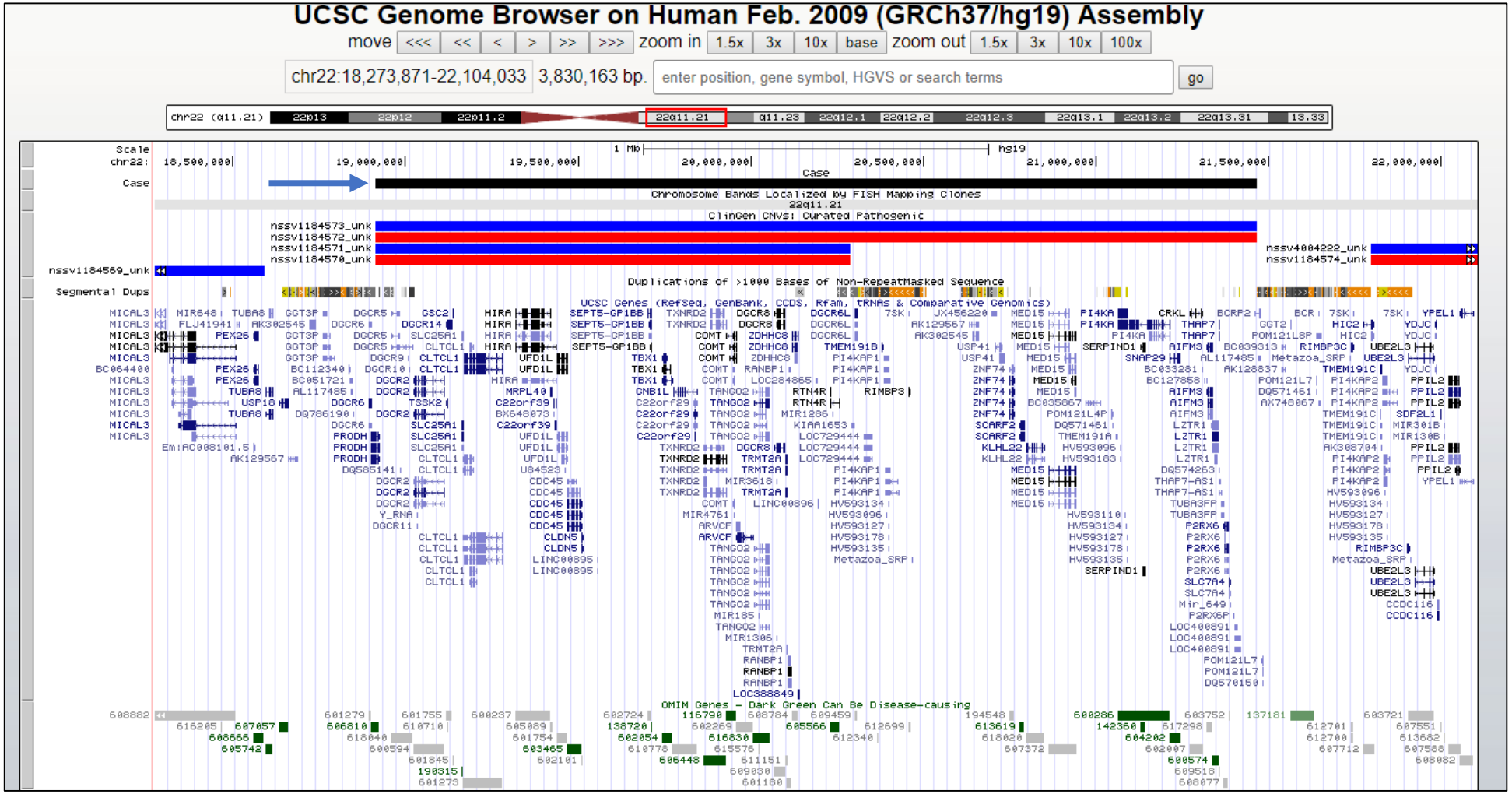
arr[GRCh37] 22q11.21(18912231_21465672)x3

- Reason for referral: 8 y/o female referred for failure to thrive, short stature, fine motor delay, gross motor delay, speech delay, learning disability, autism
- Inherited from apparently unaffected father
- Use the GAIN scoring metric

Section 1: Initial Assessment of Genomic Content

Example Case 4

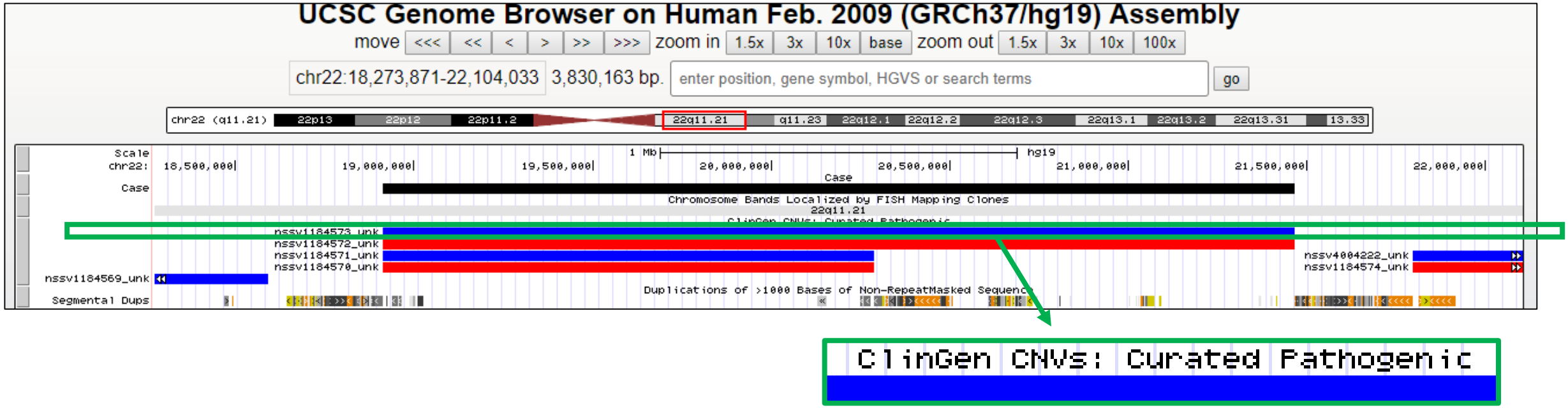
Genes (protein-coding in dark blue)



- Apply category 1A ("contains protein-coding or other known functionally important elements"), as this duplication includes many protein-coding genes
- 0 points awarded, continue evaluation

Total: 0 points

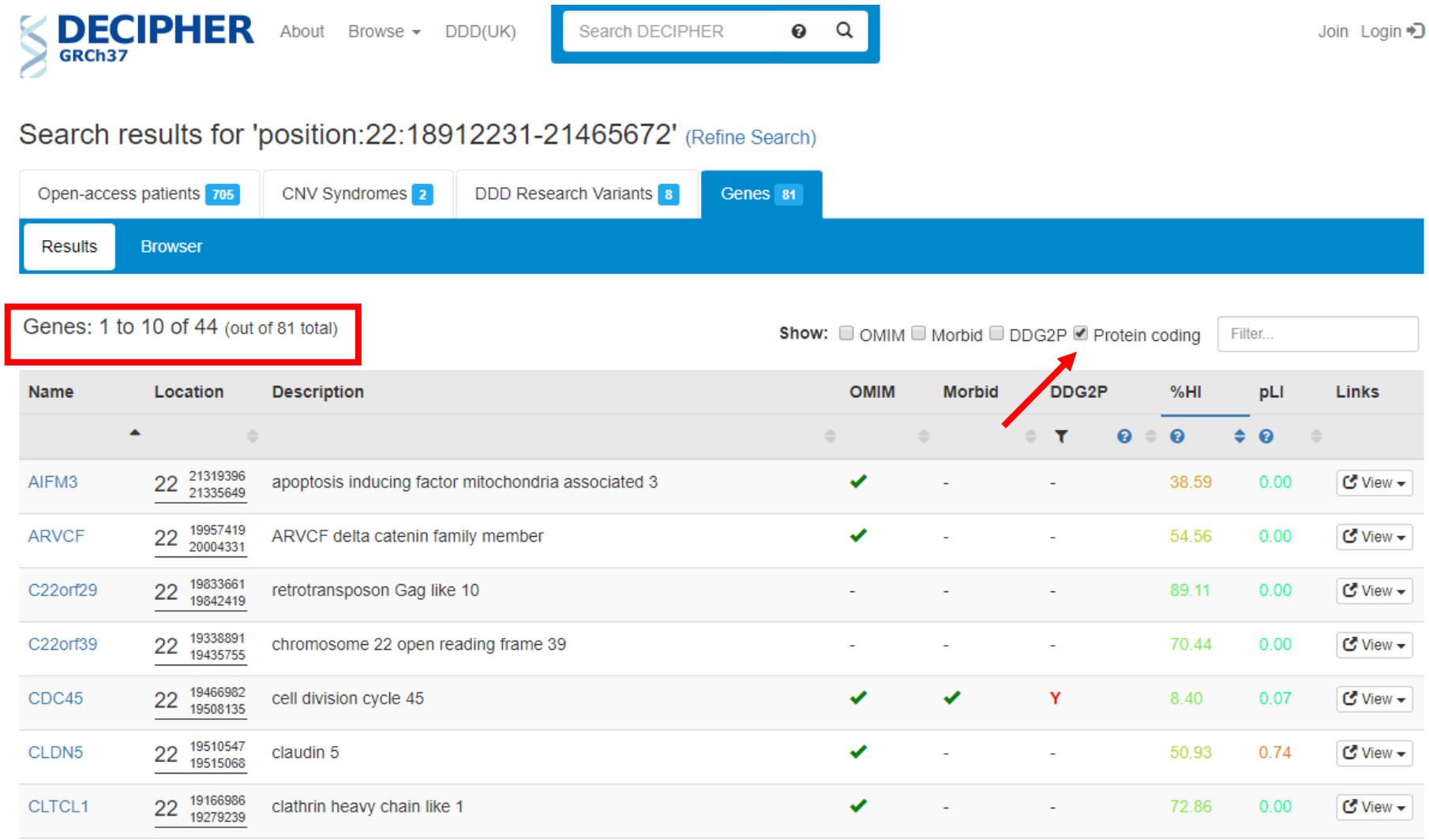
Section 2: Overlap with Established Triplosensitive (TS), Haploinsufficient (HI) or Benign Genes/Genomic Regions



- This duplication completely encompasses a ClinGen “Dosage Sensitivity 3 Score” genomic region (category 2A), a recurrent CNV mediated by rearrangements involving flanking segmental duplication regions
- This warrants 1.0 points and a classification of PATHOGENIC. Users may need not proceed further through the metric, however, for the sake of this example, we will walk through the subsequent examples.

Total: 1 point

Section 3: Evaluation of Gene Number



Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
AIFM3	22 21319396 21335649	apoptosis inducing factor mitochondria associated 3	✔	-	-	38.59	0.00	View
ARVCF	22 19957419 20004331	ARVCF delta catenin family member	✔	-	-	54.56	0.00	View
C22orf29	22 19833661 19842419	retrotransposon Gag like 10	-	-	-	89.11	0.00	View
C22orf39	22 19338891 19435755	chromosome 22 open reading frame 39	-	-	-	70.44	0.00	View
CDC45	22 19466982 19508135	cell division cycle 45	✔	✔	Y	8.40	0.07	View
CLDN5	22 19510547 19515068	claudin 5	✔	-	-	50.93	0.74	View
CLTCL1	22 19166986 19279239	clathrin heavy chain like 1	✔	-	-	72.86	0.00	View

Section 3: Evaluation of Gene Number

- 44 protein-coding genes
  - Category 3B (“35-49 genes”) → 0.45 points
- Note: multiple non-coding genes are also present in this CNV, many of which localize within segmental duplications in this region. These genes should not be counted in the evaluation of gene number.

Section 4: Detailed Evaluation of Genomic Content Using Published Literature, Public Databases, and/or Internal Lab Data

- Since this duplication completely encompasses a known triplosensitive region, and a recurrent CNV with well-characterized phenotypic variability and incomplete penetrance, sections 4A-40 would not need to be performed. We refer users to resources such as the ClinGen Dosage Sensitivity Map as a starting point to review of the literature
- NOTE: use of these metrics for recurrent regions other than those with definitive evidence classifications should be performed with caution.

Section 4, continued

- We refer users to resources such as the ClinGen Dosage Sensitivity Map as a starting point to review of the literature for established dosage sensitive genes and regions

ClinGen Dosage Sensitivity Curation Page

22q11.2 recurrent (DGS/VCFS) region (proximal, A-D) (includes TBX1)

Curation Status: Complete

id: ISCA-37446

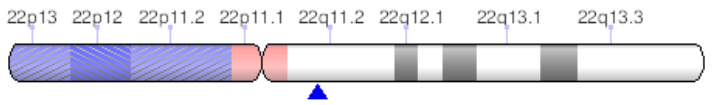
Date last evaluated: 2018-08-31

Issue Type: ClinGen Region Curation

ClinGen Haploinsufficiency Score: 3

ClinGen Triplosensitivity Score: 3

Note the date of last evaluation, particularly for reviews resulting in scores < 3. Check literature to make sure no contradictory evidence has emerged since this date.



Location Information

22q11.21

GRCh37/hg19 chr22: 18,912,231-21,465,672

View: NCBI | Ensembl | UCSC

GRCh38/hg38 chr22: 18,924,718-21,111,383

View: NCBI | Ensembl | UCSC

https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/clingen_region.cgi?id=ISCA-37446

Section 4, continued

Genome View | Evidence for Haploinsufficiency Phenotypes | Evidence for Triplosensitive Phenotypes

Triplosensitivity score: 3
Strength of Evidence (disclaimer): Sufficient evidence for dosage pathogenicity
Triplosensitivity phenotype: CHROMOSOME 22q11.2 DUPLICATION SYNDROME

Evidence for gain of function phenotype

PubMed ID	Description
20301749	Firth. GeneReviews: 22q11.2 Duplication. Review of the phenotype associated with duplication of proximal 22q11.2. Similar to the reciprocal deletion, this review combines data from reports of patients with both A-D (3 Mb) and A-B (1.5 Mb) region duplications. The phenotype is described as, ?generally mild and highly variable; findings range from apparently normal to intellectual disability/learning disability, delayed psychomotor development, growth retardation, and/or hypotonia. The high frequency with which the 22q11.2 duplication is found in an apparently normal parent of a proband suggests that many individuals can harbor a duplication of 22q11.2 with no discernible phenotypic effect.?
19254783	Portnoi (2009) reviewed the literature and summarized findings from approximately 50 unrelated cases of duplications involving proximal 22q11.2. The author noted, ?The phenotype of patients is extremely variable, ranging from multiple defects to mild learning difficulties, sharing features with DGS/VCFS, including heart defects, urogenital abnormalities, velopharyngeal insufficiency with or without cleft palate, and with some individuals being essentially normal.?
18414210	Ou et al. (2008) reviewed clinical findings of 22q11.2 CNV carriers from a cohort of 7000 clinical aCGH cases. Three probands (Pts 1-3) and two relatives of patient 1 (sibling and mother) with A-D (3 Mb) region duplications were reported. Clinical findings in common in at least two carriers included developmental delay, speech delay, variable dysmorphic features, hypernasal speech, hearing impairment, and behavioral abnormalities. Parental samples were unavailable for patients 2 and 3.

Triplosensitivity phenotype comment:

Duplication of the 22q11.2 proximal (A-D) region* is associated with a highly variable clinical phenotype, ranging from apparently normal to expression a broad range of clinical features, including nonspecific phenotypes (intellectual disability, learning disability, developmental delays, autism, psychiatric disorder growth delays, hypotonia) as well as phenotypes that overlap clinical findings of DGS/VCFS. 22q11.2 duplications are frequently inherited; incomplete penetrance has been demonstrated. This duplication is enriched in the clinical population.

*The 22q11.2 region contains a cluster of low copy repeats (LCRs) that mediate recurrent copy number changes through non-allelic homologous recombination. Proximal 22q11.2 rearrangements involve the centromeric LCR22A and either LCR22B (A-B, approximate size 1.5 Mb) or LCR22D (A-D, approximate size 3.0 Mb) (note in some literature these LCRs are referred to as LCR22-2, 3a and -4, respectively). This review refers to patients with the more common A-D rearrangement. The phenotypes in individuals with A-B and A-D duplications are largely indistinguishable. Note that genes used as landmarks are not necessarily causative of the phenotype(s) associated with the region.

Additional relevant literature is summarized below:

Section 5: Evaluation of Inheritance Patterns/Family History of Patient Being Studied

- Inherited from apparently unaffected father
  - Reported phenotype (failure to thrive, short stature, fine motor delay, gross motor delay, speech delay, learning disability, autism) is relatively non-specific
  - Category 5C, 0 points (see NOTE)
- NOTE: for any CNV that has well-documented phenotypic variability and/or incomplete penetrance, including many recurrent CNVs, this section of the metrics will not apply and should not be used to add or deduct points from an already classified CNV (for example, even if this 22q11.2 duplication was inherited from an unaffected parent, we should not deduct points as incomplete penetrance is established). The points ranges allow one to assign 0 points for such cases.

- Total points: 1 (from Section 2A); Proposed Classification: Pathogenic