ClinGen
Clinical Genome Resource

Defining the clinical relevance of genes & variants for precision medicine and research...

ACMG/ClinGen Constitutional CNV Technical Standards Web Series: Series Participation Information

Hosted by ClinGen

Welcome!

- Goal: to familiarize the community with the new ACMG/ClinGen technical standards for interpretation and reporting of constitutional CNVs
  - In-depth review of different pieces of the scoring metrics
  - Examples
  - Q&A

Date	Topic	Presenter
January 16, 2020	Overview: Updated Technical Standards for Constitutional CNVs	Erin Rooney Riggs (Geisinger)
January 23, 2020	Use of the ClinGen Dosage Sensitivity Map	Erica Andersen (ARUP)
January 30, 2020	Scoring Case-Level Data	Erin Rooney Riggs (Geisinger)
February 6, 2020	Special Considerations in the Evaluation of Intragenic CNVs	Daniel Pineda-Alvaraz (Invitae)
February 13, 2020	Understanding Case-Control Data	Bradley Coe (BC Children's and BC Women's Hospitals)
February 20, 2020	Resources for Population CNV Data: gnomAD SV	Ryan Collins (Broad Institute)
February 27, 2020	Resources for Population CNV Data: Database of Genomic Variants (DGV)	Jeff MacDonald (The Hospital for Sick Children)
March 5, 2020	Uncoupling Variant Classification from Clinical Significance: Considerations for Reporting	Christa Lese Martin (Geisinger)
March 12, 2020	General Questions and Answers	All

Logistics

- As of January 15, over 600 people have registered to attend this web series
  - 36 different countries
  - Many different professional roles (laboratory directors, laboratory staff, genetic counselors, researchers, physicians, fellows/trainees, variant scientists, bioinformaticians, etc.)
- Anyone can join the web series at any time
  - Please complete the registration survey so we can track metrics on attendees
  - ***https://tinyurl.com/CNVRegistration***
- All webinars will be recorded and posted to www.clinicalgenome.org and the ClinGen YouTube channel ("ClinGen Resource")
  - Slides will also be made available

Questions

- Because of the large number of participants and the fact that we will be recording each webinar, all participants will be MUTED upon entry
- If you have a question:
  - Type it into the Q&A box – we will be monitoring this throughout each webinar
    - You will not be able to do this if you are a call-in user only – please sign in on your computer
  - Questions will be addressed at the end of each webinar, time permitting
    - Please make sure your full name is listed so we can follow up with you if needed (e.g., do not simply type in your first name, or initials, etc.)
    - Questions not answered on a particular webinar will be saved for the Q&A session on March 12
  - Feel free to email questions at any time to clingen@clinicalgenome.org

Attendance

- In addition to tracking interest through the registration survey, we will also be tracking how many people actually attend each webinar
- An attendance survey URL and QR code will be shown at the beginning of each webinar and included in the chat.
  - Please take a moment to fill this out!
  - This will allow us to demonstrate the reach of this program, identify which topics generated the most interest, etc.
  - Also an important variable in our pre-/post-series CNV evaluation study
- Please also fill out the attendance survey if you are unable to attend live but watch a video at a later date.

Today’s Attendance URL and QR code:

https://tinyurl.com/AttendanceJan16

Pre- and Post-Series CNV Evaluation Project

- Optional project offered to people registering for the web series prior to December 19, 2019
  - 254 potential participants (enrollment now closed)
  - Divided into 4 groups
- Evaluate 5 CNVs before the start of the series (by Jan 15), and 5 CNVs after the conclusion of the web series (by April 9)
  - Goals: to determine whether or not education improved CNV evaluation skills; identify content areas that may require more targeted education/resources
- Assignments have been emailed to you; please submit results via applicable SurveyMonkey surveys
- Attendance tracking is especially important for this group; this will be a variable in our analysis
- Questions? Email: clingen@clinicalgenome.org

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Defining the clinical relevance of genes & variants for precision medicine and research...

Overview: Technical Standards for the Interpretation and Reporting of Constitutional Copy Number Variants: An ACMG/ClinGen Joint Consensus Recommendation

Erin Rooney Riggs, MS, CGC
Assistant Professor, Autism & Developmental Medicine Institute
Geisinger - Danville, Pennsylvania, USA
eriggs@geisinger.edu

(On behalf of the ACMG/ClinGen Copy Number Variant Interpretation Working Group)

Laboratories can be discordant in the interpretation of the *same* genomic variant

- Genomic variant interpretation requires the synthesis and evaluation of evidence from a variety of sources – resulting in a subjective process
- Discordant interpretation is a problem for both sequence and copy number variants:
  - Documented in the literature (Tsuchiya *et al*. 2009, Amendola *et al*. 2016, etc.)
  - As of January 2020, ~4.6% of variants have conflicts among unique variant submissions with interpretations in ClinVar
- Interpretation guidelines were developed for both groups to help guide laboratories toward more consistent interpretations

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

Genetics in Medicine • Volume 13, Number 7, July 2011

Original CNV interpretation guidelines provide guidance on the types of evidence to consider, but no specific recommendations on the relative importance of each to the overall evaluation

Time to update

- Original guidelines put forth at a time when CMA was just coming in to wide clinical use
- More data and experience allows us to refine previous recommendations
- New technologies are requiring us to think beyond just CMA
- Ultimate goal: consistent CNV interpretation
  - Across labs
  - Across technologies
  - Across specialties

Summary of Major Changes

- New technical standards officially released November 6, 2019
- Major changes include:
  - Officially adopt five-tier classification system
  - “Uncouple” variant classification from clinical interpretation
  - Incorporate a quantitative, evidence-based evaluation framework

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ACMG TECHNICAL STANDARDS | Genetics inMedicine

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]

Officially Adopt the 5-Tier Variant Classification System

Current

- Pathogenic
- Uncertain Clinical Significance
  - Uncertain clinical significance, likely pathogenic
  - Uncertain clinical significance
  - Uncertain clinical significance, likely benign
- Benign

Proposed

- Pathogenic
- Likely Pathogenic
- Uncertain Significance
- Likely Benign
- Benign

“Uncouple” Variant Classification from Clinical Significance

More on March 5!

- 2 different concepts:
  - Variant classification: Do we have enough evidence to say that this variant causes disease?
  - Clinical Significance: Is this variant causing disease *in my particular patient?*

- Examples:
  1. LOF of a particular gene on the X chromosome is known to be associated with disease. You observe a deletion of this gene in a male case, and a similar deletion in a female case.
  2. LOF of a particular gene is associated with hearing loss. You observe a deletion of this gene in a case referred for hearing loss, as well as in a case referred for failure to thrive.

Classification vs. Clinical Significance

- In both scenarios, the CLASSIFICATION of the variant should remain the same
  - The evidence supporting these classifications (at the same point in time) is the same
  - Therefore, the variant should receive the same CLASSIFICATION (P, LP, VUS, LB, or B) regardless of the context in which it is observed
- In each scenario, the CLINICAL SIGNIFICANCE of the variant could be different
  - In scenario 1 (X-linked), the variant could be disease-causing in a hemizygous male, but represent a carrier state in a heterozygous female.
  - In scenario 2 (RFR), the variant could explain one patient's RFR, but represent a incidental finding in the other
- Clearly labeled sections on a report delineating findings related to the RFR, findings that may be incidental, and findings that may represent carrier status could be utilized to make this clear

Classification vs. Clinical Significance

- Why is this important?
  - Major source of discrepancy between laboratories
    - Variants involving the X chromosome represented almost 20% of the potential conflicts flagged in a recent analysis of CNVs overlapping known dosage sensitive genes in ClinVar (Riggs *et al.* 2018)
    - Almost all of these (85%) were due to inconsistencies in interpretation of these types of variants for female patients (B to P)
    - Anecdotally, we are aware of laboratories interpreting variants as VUS when the associated disease does not match their patient's RFR, regardless of the level of evidence available to support the gene/disease relationship
  - A given CNV should be classified the same way regardless of whether it is observed on CMA or using sequencing-based technologies

Quantitative, Evidence-Based Evaluation Framework

- Aims:
  - Provide more specific guidance to users regarding the weights of particular pieces of evidence to increase inter-laboratory consistency
  - Align interpretation recommendations (where possible) between CNV and sequence variant interpretation guidelines published by ACMG/AMP in 2015

Independent scoring metrics for deletions and duplications

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

- A suggested number of points are added or subtracted per each piece of evidence.
- Point values assigned based on evidence strength.
- The total number of points guides the user to a *preliminary* classification

Suggested point values roughly correspond to ACMG/AMP Sequence Guideline Evidence Strengths

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

Combining rules are similar (e.g. 3 Moderate (0.30) = LP (0.90); 1 Very Strong (0.90) + ≥2 Moderate (0.30) = P (>0.99), etc.)

Useful resource: Tavtigian *et al*. 2018 (PMID: 29300386)

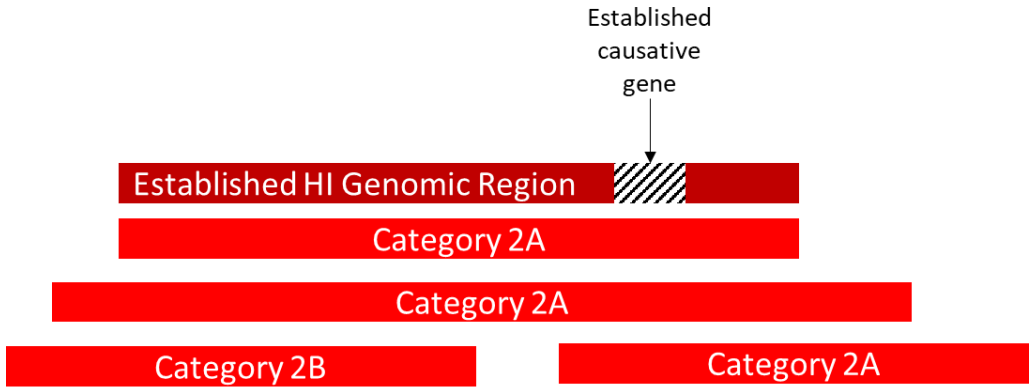
Section 1: Initial Assessment of Genomic Content

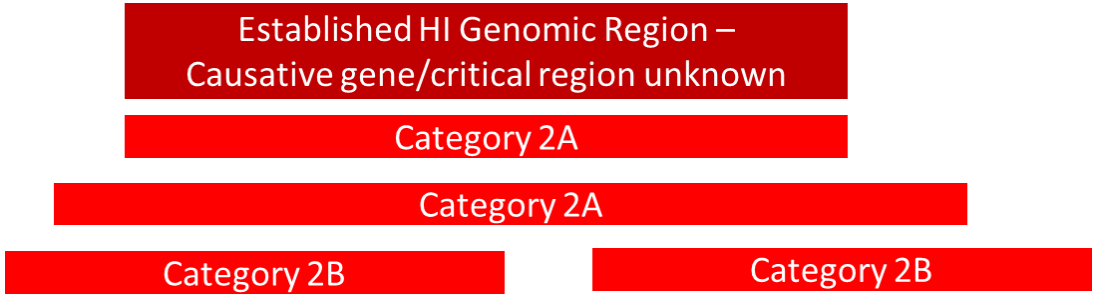
Section 1: Initial Assessment of Genomic Content				
Evidence Type	**Evidence**	**Suggested Points/Case**	**Max Score**	**Points Given**
Copy number loss content (For intragenic variants, use section 4)	**1A**. Contains protein-coding or other known functionally important elements	0 (Continue Evaluation)	0	
	1B. Does NOT contain protein-coding or any known functionally important elements[1]	-0.60	-0.60	

Section 2: Overlap with Established HI Genes/Genomic Regions

More on Jan 23!

Section 2: Overlap with Established/Predicted HI or Established Benign Genes/Genomic Regions *(Skip to Section 3 if your copy number loss DOES NOT overlap these types of genes/regions)*			
	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





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

NCBI

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ClinGen Dosage Sensitivity Map

The Clinical Genome Resource (ClinGen) consortium is curating genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage sensitive and should be targeted on a cytogenomic array.

All data are shown in GRCh37 and GRCh38 coordinates.

Search By Gene Name

Symbol:

Or click on the following examples: ZEB2, PTEN, MAPT

Go

Search By Location (GRCh37)

Location:

example: chr2:44,000,000-45,500,000, 2p21-2p16.2

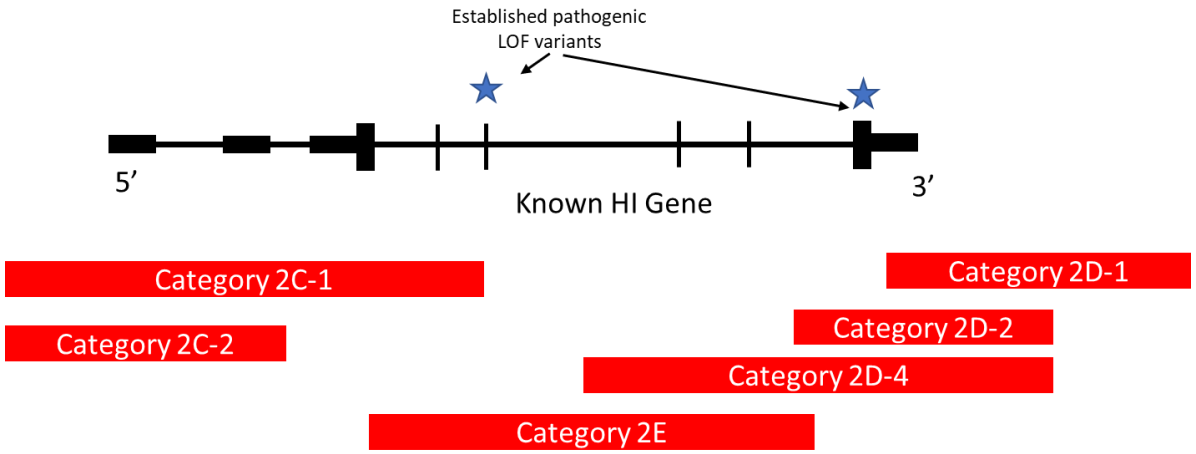
Go

Links

- ClinGen Home Page
- Help with this site
- FAQ
- Contact Us
- Pathogenic CNV regions
- Curation of the ACMG 59 Genes
- FTP

Section 2: Overlap with Established HI Genes/Genomic Regions

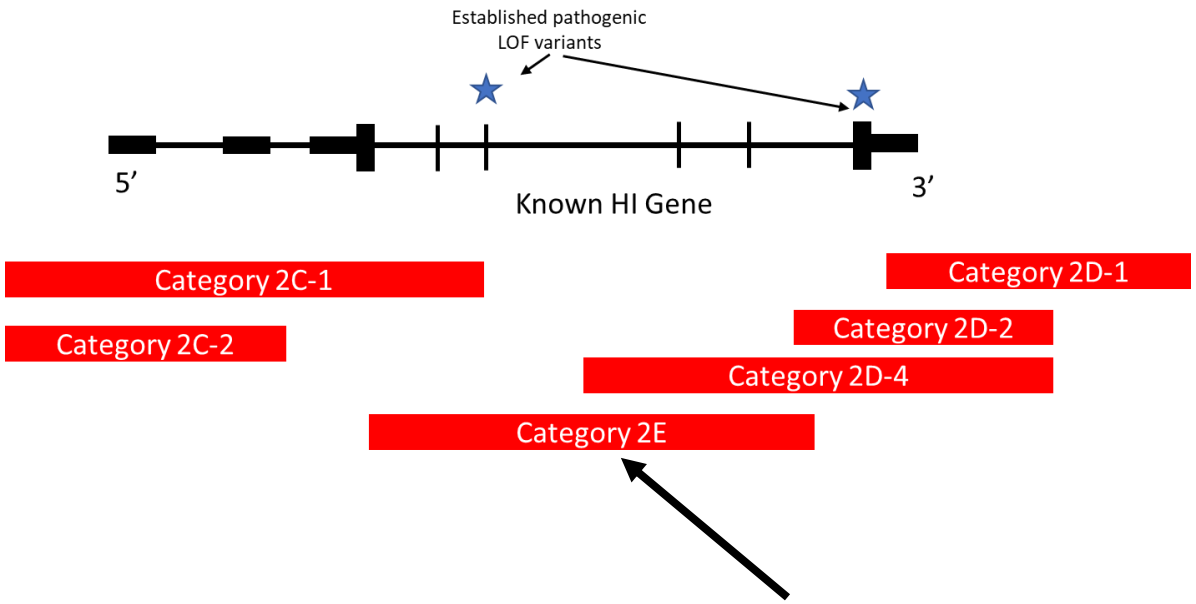
2C. Partial overlap with the 5' end of an established HI gene (3' end of the gene not involved)…	See categories below		
2C-1. …and coding sequence is involved	0.90 *(Range[2]: 0.45 to 1.00)*	1.00	
2C-2. …and only the 5' UTR is involved	0 *(Range[2]: 0 to 0.45)*	0.45	
2D. Partial overlap with the 3' end 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[2]: 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.[4]	0.30 (Range[2]: 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[2]: 0.45 to 1.00)	1.00	



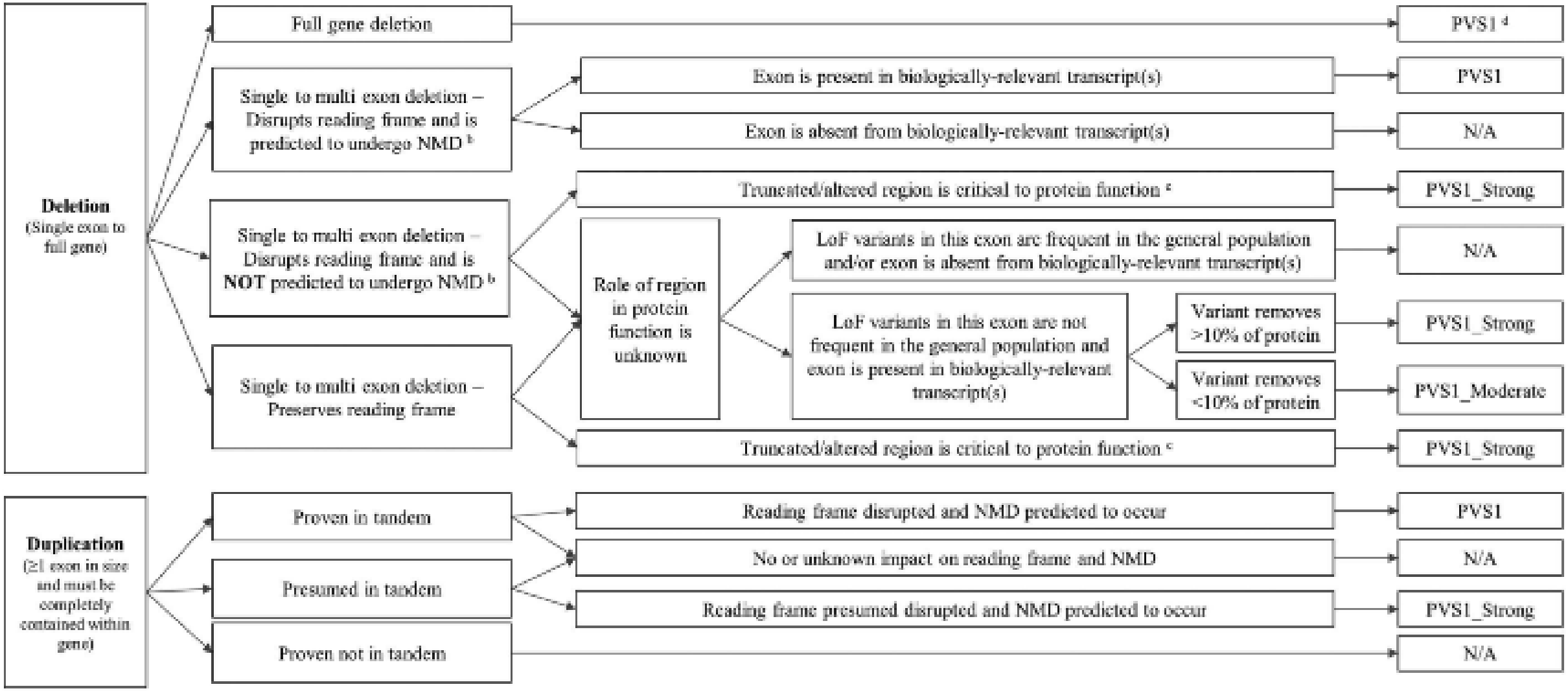
More on Feb 6!

Section 2: Overlap with Established HI Genes/Genomic Regions

2E. Both breakpoints are within the same gene (intragenic CNV; gene-level sequence variant)[5]	See ClinGen SVI working group PVS1 specifications[6] • PVS1= 0.90 *(Range[2]: 0.45 to 0.90)* • PVS1_Strong= 0.45 *(Range[2]: 0.30 to 0.90)* • PVS1_Moderate *or* PM4 (in-frame indels) = 0.30 *(Range[2]: 0.15 to 0.45)* • PVS1_Supporting = 0.15 *(Range[2]: 0 to 0.30)* • N/A = No points, but continue evaluation	See categories at left	



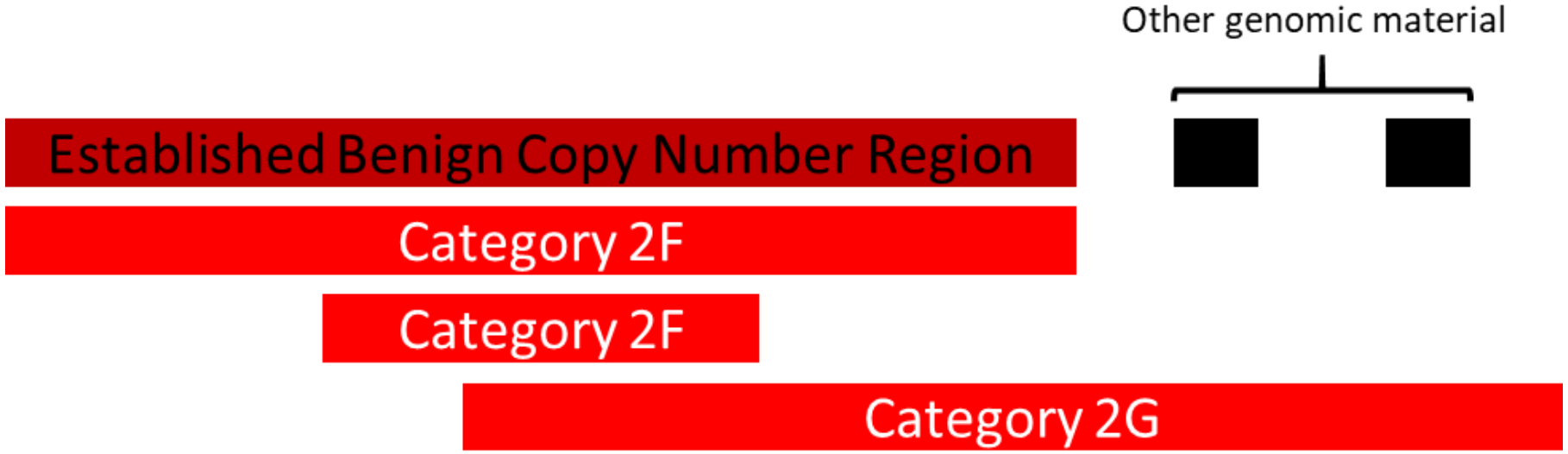
What about intragenic variants?



Abou Tayoun *et al.* 2018 (PMID: 30192042)

Section 2: Overlap with Established Benign Genes/Genomic Regions

Overlap with ESTABLISHED benign[7] 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[8]	0 (Continue evaluation)	0	

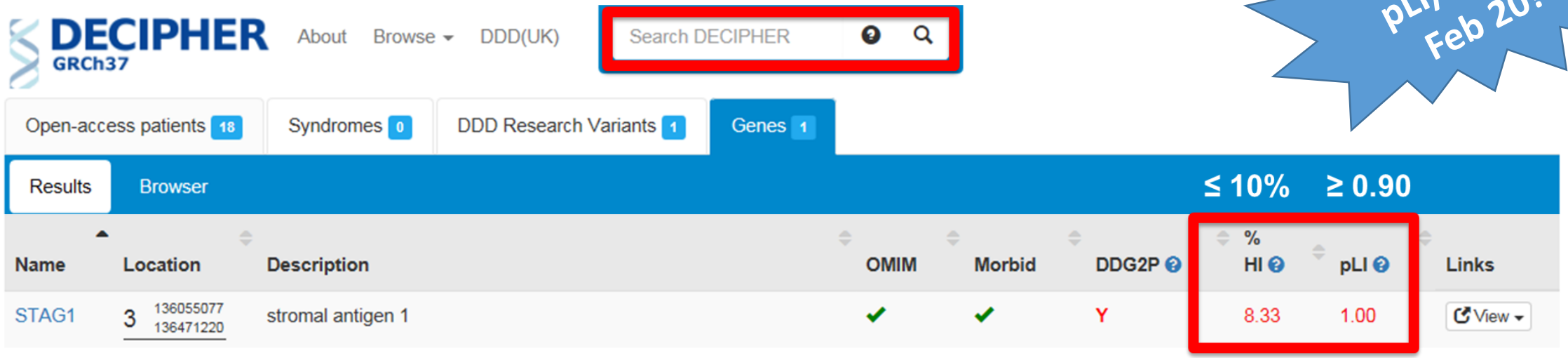


Section 2: Haploinsufficiency Predictors

Haploinsufficiency Predictors	**2H.** Multiple HI predictors[9] suggest that AT LEAST ONE gene in the interval is haploinsufficient (HI)	0.15	0.15	

- Analogous to "Computational Data" category in ACMG/AMP sequence variant guidelines – lower level evidence
- When multiple predictors agree (for at least 1 gene in the CNV interval), this can be considered a low-weight piece of supportive evidence
- Current recognized HI predictors – DECIPHER HI, gnomAD pLI/LOEUF

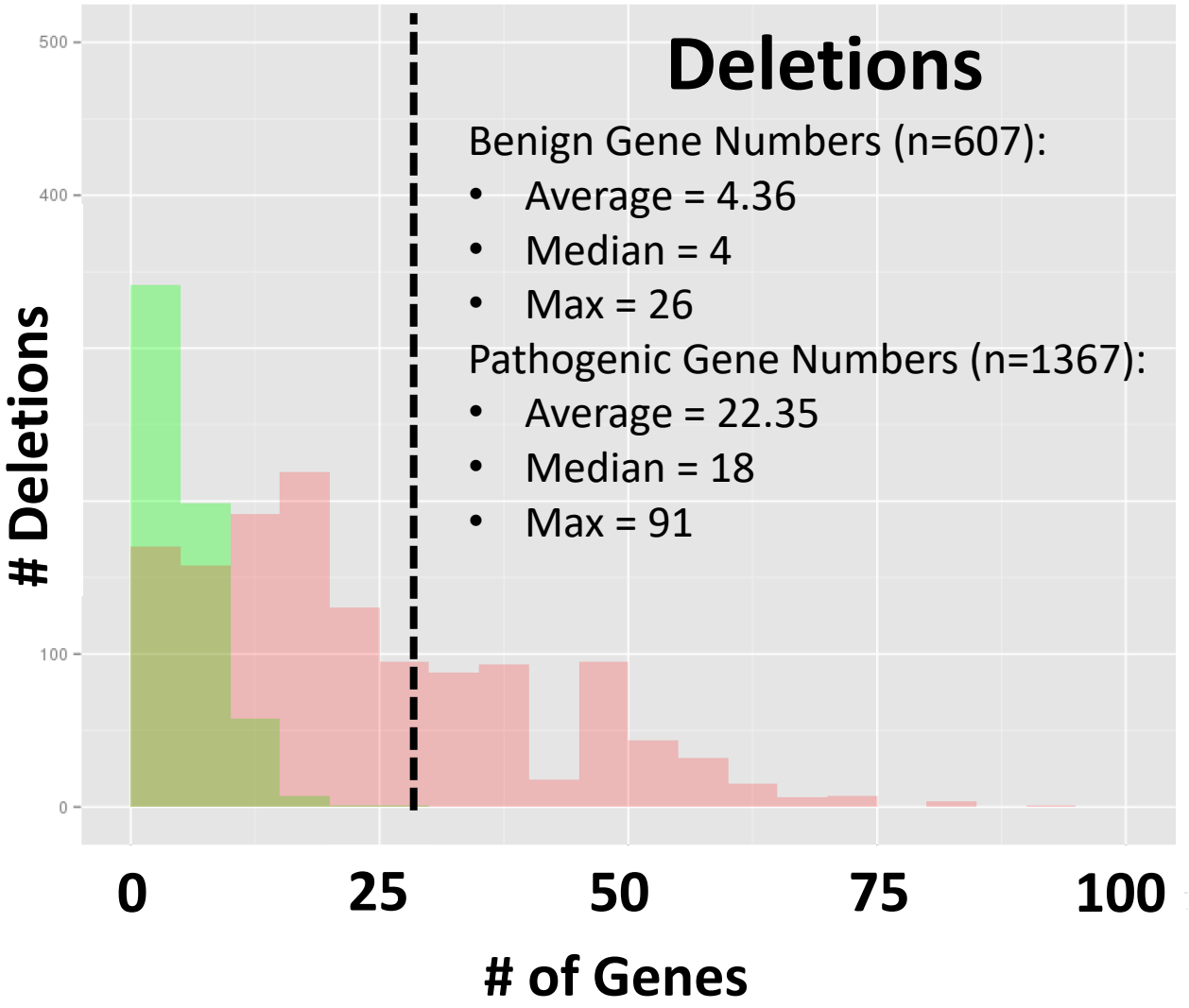
More on pLI/LOEUF Feb 20!

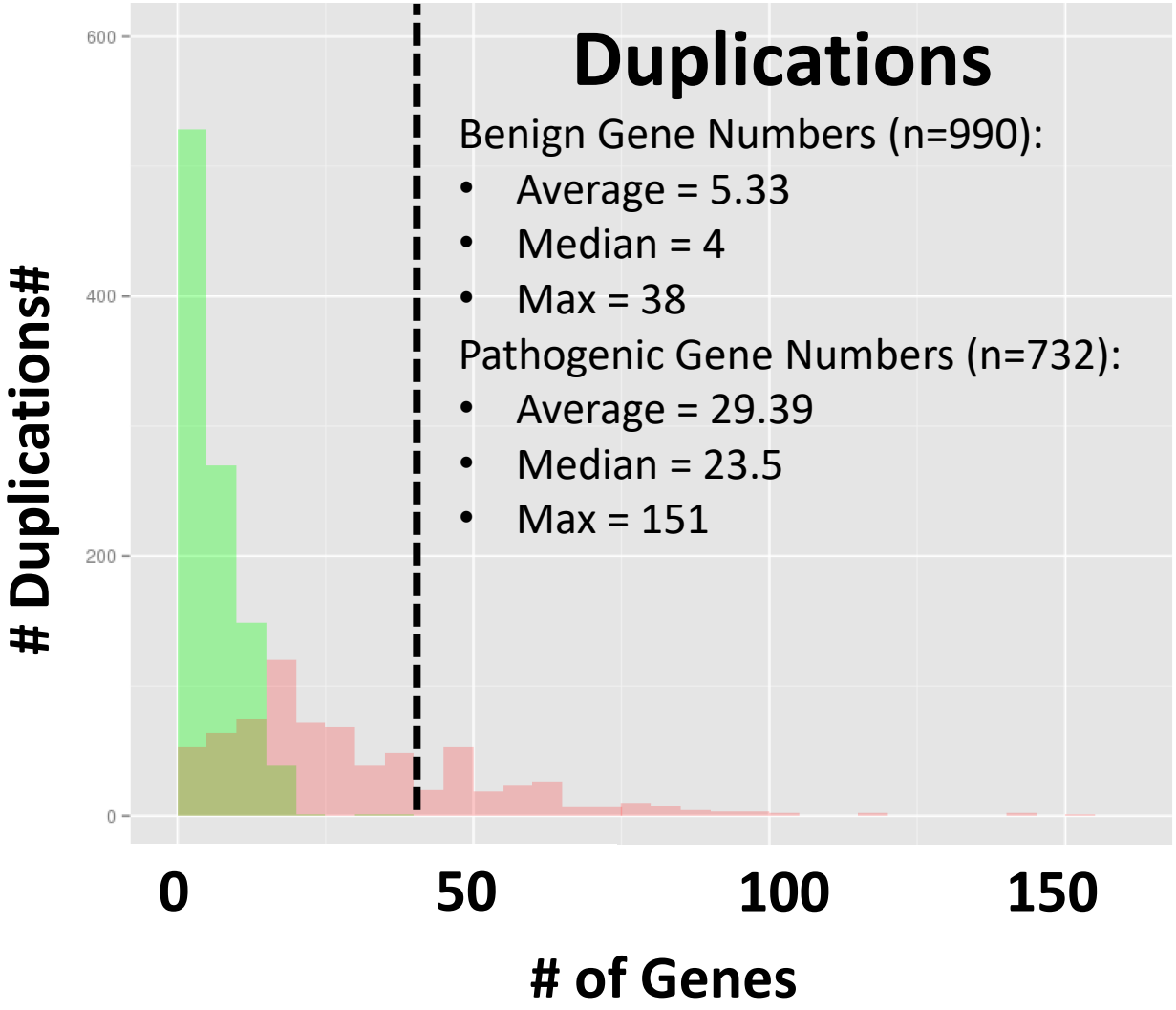


Section 3: Evaluation of Gene Number

Section 3: Evaluation of Gene Number				
Number of protein-coding RefSeq genes wholly or partially included in the copy number loss[10]	3A. 0-24 genes	0	0	
	3B. 25-34 genes	0.45	0.45	
	3C. 35+ genes	0.90	0.90	

- IN GENERAL, larger CNVs are more likely to be interpreted as Pathogenic (P) or Likely Pathogenic (LP)
  - Cytogenetically visible
  - Large number of genes
  - Absolutely exceptions to this generalization!
- When there is no other information available for a CNV, when does the sheer size prompt one to classify it as P or LP?
- Can we identify a conservative threshold for gene number, above which it is unlikely that a CNV would be interpreted as anything other than P?





Classified as “Benign” Classified as “Pathogenic”

Analysis by Deborah Ritter, PhD

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

Section 4: Detailed Evaluation of Genomic Content Using Published Literature, Public Databases, and/or Internal Lab Data[11] *(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*[12]	Reported proband 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[12] *de novo*: 0.45 points each Assumed[11] *de novo*: 0.30 points each *(Range[2]: 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[12] *de novo*: 0.30 points each Assumed[11] *de novo*: 0.15 point each *(Range[2]: 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[12] *de novo*: 0.15 point each Assumed[12] *de novo*: 0.10 point each *(Range[2]: 0 to 0.30)*		
	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[2]: 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[2]: 0 to 0.15)	0.30 (total)	

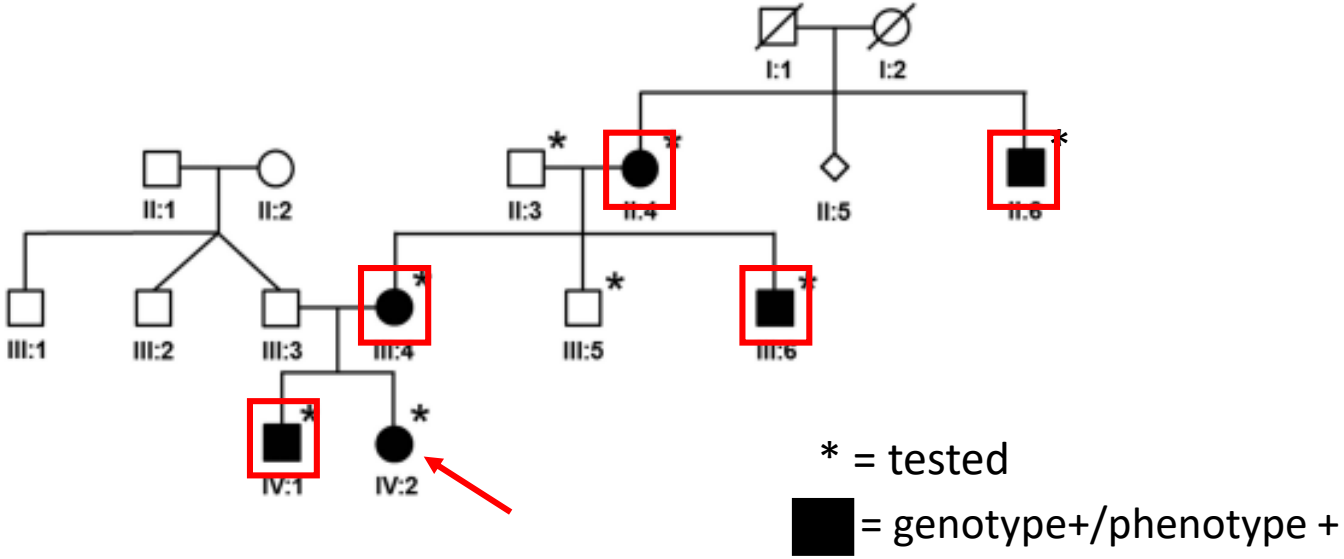
- Highly specific, relatively unique:
  - Fixed, dilated pupils (Gillespie syndrome)
  - Fetal adrenocortical cytomegaly (Beckwith-Wiedemann)
- Highly specific, not necessarily unique
  - Early infantile epileptic encephalopathy (68 entries in OMIM)
  - Spastic paraplegia (68 entries in OMIM)
- Not highly specific, and/or with high genetic heterogeneity
  - Developmental delay/intellectual disability
  - Autism

More on Jan 30!

Section 4: Case evidence, Segregation In Affected Family Members

Individual case evidence – segregation among similarly affected family members[13]	4F. 3-4 observed segregations	0.15	0.45	
	4G. 5-6 observed segregations	0.30		
	4H. 7 or more observed segregations	0.45		

- For simplicity, count only genotype +/phenotype + individuals, and/or obligate carriers



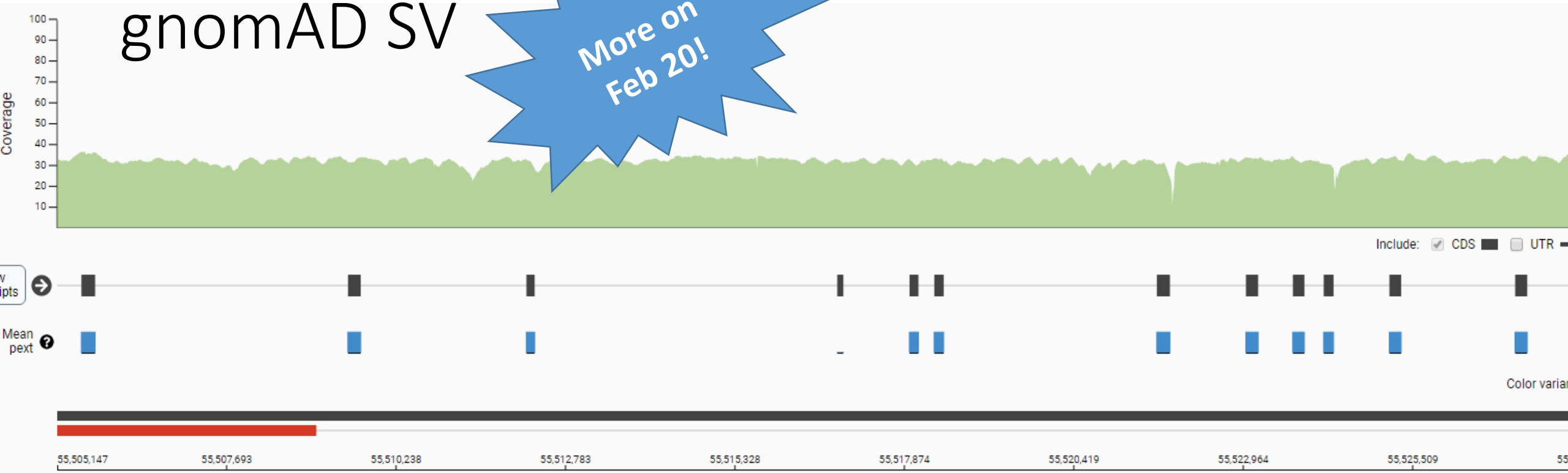
Section 4: Case-Control and Population Evidence

Case-control and population evidence[15]	**4L.** Statistically significant increase amongst observations in cases (**with a consistent, specific, well-defined phenotype**) compared to controls	0.45 per study *(Range[2]: 0 to 0.45 per study)*	0.45 (total)	
	4M. Statistically significant increase amongst observations in cases (**without a consistent, non-specific phenotype OR unknown phenotype**) compared to controls	0.30 per study *(Range[2]:0 to 0.30 per study)*	0.45 (total)	
	4N. No statistically significant difference between observations in cases and controls[16]	-0.90 (per study) *(Range[2]:0 to -0.90 per study)*	-0.90 (total)	
	4O. Overlap with common population variation[17]	-0.90 *(Range[2]:0 to -0.90)*	-0.90	

More on case-control Feb 13!

gnomAD SV

More on Feb 20!



Consequences

LoF only | Int. exon duplication only | Copy gain only | Other only

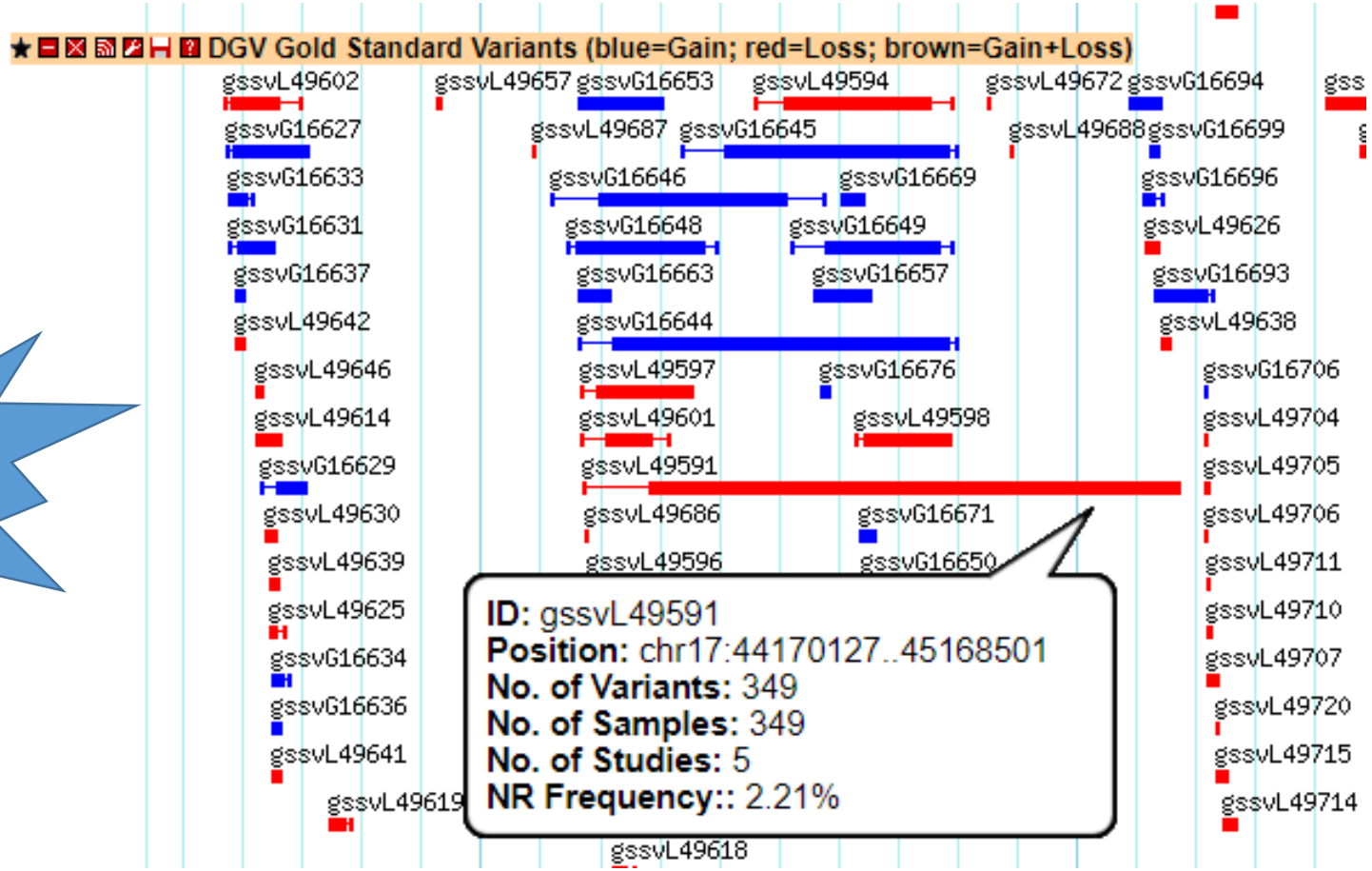
Include filtered variants

Classes

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

Variant ID	Source	Consequence	Class	Position	Size	Allele Count	Allele Number	Allele Frequency
INV_1_7	G	inversion span	inversion	35173490 - 93458530	58.3 Mb	1	21476	4.7e-5
DEL_1_4654	G	loss of function	deletion	55494890 - 55509041	14.2 kb	2	21476	9.3e-5

Database of Genomic Variants (DGV)



Section 5: Inheritance/Family History for Patient Being Studied

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[2]: 0 to -0.45)	-0.45	
	5C. Patient with **non-specific** phenotype and no family history. CNV is inherited from an apparently unaffected parent.	-0.15 (Range[2]: 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 – NON-SEGREGATIONS	**5E.** *Use appropriate category from non-segregation section in Section 4.*	*Use non-segregation 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 non-specific, but is consistent with what has been described in similar cases.[18]	0.10 (Range[2]: 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.[18]	0.30 (Range[2]: 0 to 0.30)	0.30	

CNV Calculator

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

Pathogenic
Total score: 1.1

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ClinGen CNV Pathogenicity Calculator

CNV Interpretation Scoring Rubric: Copy Number LOSS

Evidence Type	Evidence	Suggested points	Max Score	Points Given
Section 1: Initial Assessment of Genomic Content				
Copy number loss content (For intragenic variants, use section 4)	☑ 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	-0.75	Assigned points: 0
Section 2: Evaluation of Gene Number				
Number of protein-coding RefSeq genes wholly or partially included in the copy number loss	☑ 2B. 15-34 genes	0.15	0.15	Select From Menu Assigned points: 0
Section 3: Evaluation of Haploinsufficiency (HI) Predictors				
Section 3: Evaluation of Haploinsufficiency (HI) Predictors	☑ HI Predictors	0.15	0.15	Assigned points: 0.15
Section 4: Overlap with Established HI or Benign Genes or Genomic Regions *(Skip to Section 5 if your copy number loss DOES NOT overlap these types of genes/regions)*				
	☑ 4A. Complete overlap of an established HI gene/genomic region OR Partial overlap of an established HI genomic region, resulting in loss of the known causative gene or critical region	1	1	Assigned points: 0.95
	☐ 4B. 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	0	0	

Summary

- The updated technical standards intend to provide guidance to help improve consistency and increase transparency in the process of constitutional CNV classification.

Acknowledgements

- Christa Lese Martin
- Daniel Pineda Alvarez
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- Deborah Ritter
- Sarah South
- Erik Thorland
- Outside Reviewers (11)
- ClinGen Genomic Variant and Sequence Variant Interpretation Working Groups
- Ronak Patel and ClinGen Baylor team for CNV calculator

ClinGen is primarily funded by the National Human Genome Research Institute (NHGRI), through the following three grants:U41HG006834, U41HG009649, U41HG009650

Today’s Attendance URL and QR code:

https://tinyurl.com/AttendanceJan16

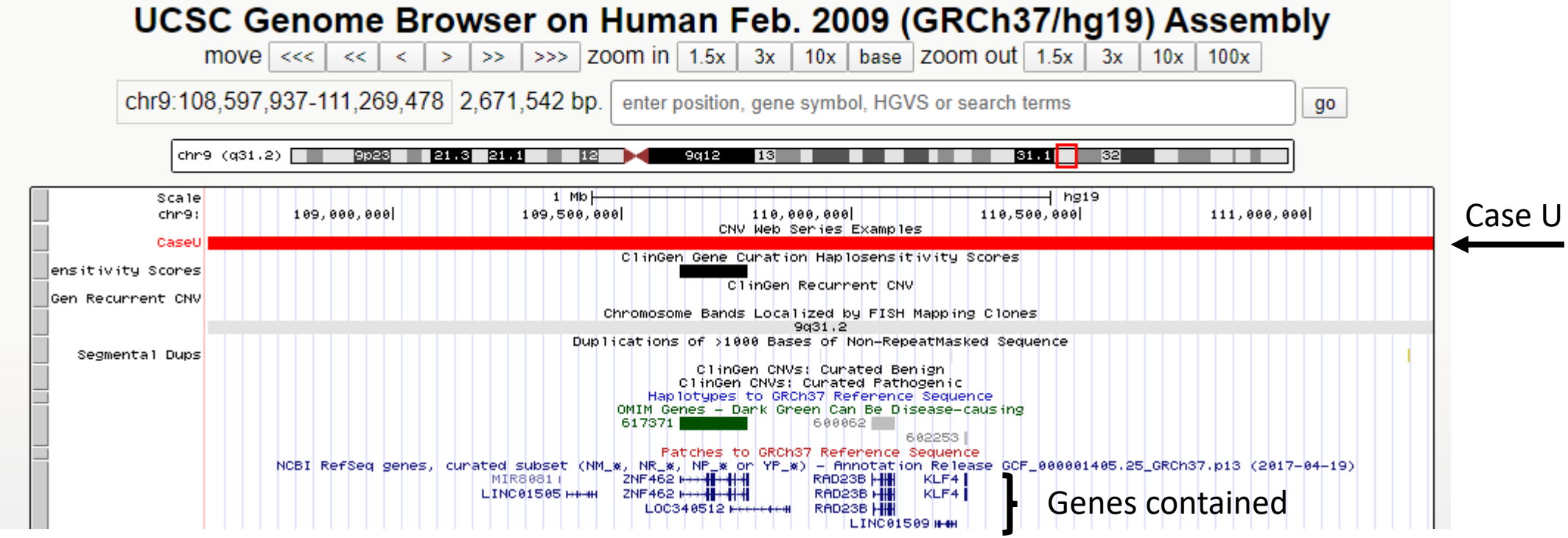
Case U
arr[hg19] 9q31.2 (108,597,937-111,269,478) x 1

2 year-old female referred for developmental delay, dysmorphic features (unspecified), and history of ventricular septal defect; inheritance unknown

Clinical Information

- arr[hg19] 9q31.2 (108,597,937-111,269,478) x 1
- 2 year-old female referred for developmental delay, dysmorphic features (unspecified), and history of ventricular septal defect
- Inheritance is unknown
- Use the LOSS scoring metric

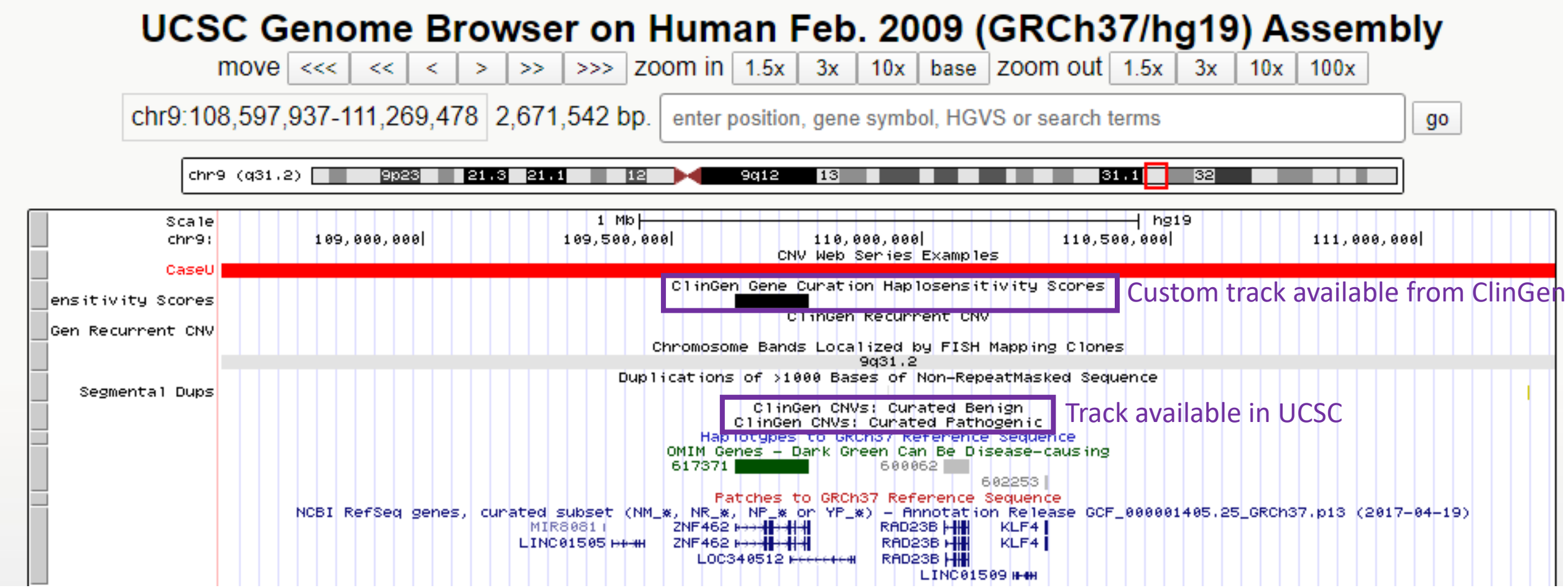
Section 1: Initial Assessment of Genomic Content



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

Total: 0 points

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



Dosage Sensitivity Tracks - UCSC

- UCSC offers a Dosage Sensitivity track within their browser
- Find it under “ClinGen CNVs” in the “Phenotype and Literature” track section
- ONLY shows genes/regions curated as “Sufficient Evidence” for dosage sensitivity or “Dosage Sensitivity Unlikely”

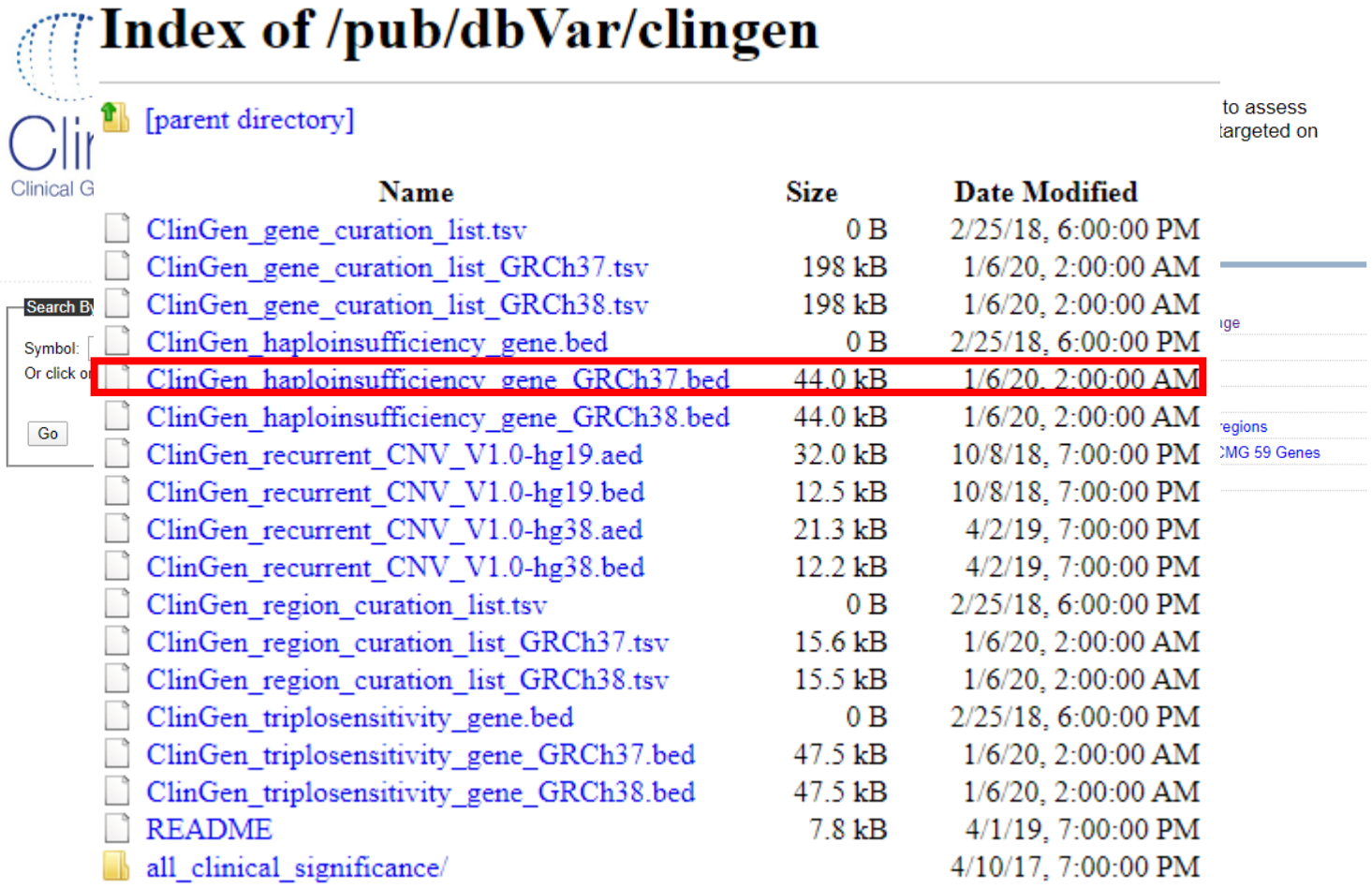
List subtracks: only selected/visible all (2 of 11 selected)

		Phenotype ↓1	Confidence ↓2	views ↓3	Track Name ↓4	
☑	pack	Benign	Curated	CNVs	ClinGen CNVs: Curated Benign	schema
☐	full	Benign	Determined	Total	ClinGen CNVs: Benign Gain Total	schema
☐	full	Benign	Determined	Total	ClinGen CNVs: Benign Loss Total	schema
☐	pack	Benign	Determined	CNVs	ClinGen CNVs: Benign	schema
☐	pack	Benign	Likely	CNVs	ClinGen CNVs: Uncertain: Likely Benign	schema
☑	pack	Pathogenic	Curated	CNVs	ClinGen CNVs: Curated Pathogenic	schema
☐	full	Pathogenic	Determined	Total	ClinGen CNVs: Pathogenic Gain Total	schema
☐	full	Pathogenic	Determined	Total	ClinGen CNVs: Pathogenic Loss Total	schema
☐	pack	Pathogenic	Determined	CNVs	ClinGen CNVs: Pathogenic	schema
☐	pack	Pathogenic	Likely	CNVs	ClinGen CNVs: Uncertain: Likely Pathogenic	schema
☐	pack	Unknown	Uncertain	CNVs	ClinGen CNVs: Uncertain	schema

2 of 11 selected

Dosage Sensitivity Tracks: ClinGen

- ClinGen offers custom tracks you can upload into the browser of your choice through the Dosage Sensitivity website
- Shows any gene/region that has been curated, regardless of score
- Good visual cue to check the Dosage site for additional information



Index of /pub/dbVar/clingen

[parent directory]

Name	Size	Date Modified
ClinGen_gene_curation_list.tsv	0 B	2/25/18, 6:00:00 PM
ClinGen_gene_curation_list_GRCh37.tsv	198 kB	1/6/20, 2:00:00 AM
ClinGen_gene_curation_list_GRCh38.tsv	198 kB	1/6/20, 2:00:00 AM
ClinGen_haploinsufficiency_gene.bed	0 B	2/25/18, 6:00:00 PM
ClinGen_haploinsufficiency_gene_GRCh37.bed	44.0 kB	1/6/20, 2:00:00 AM
ClinGen_haploinsufficiency_gene_GRCh38.bed	44.0 kB	1/6/20, 2:00:00 AM
ClinGen_recurrent_CNV_V1.0-hg19.aed	32.0 kB	10/8/18, 7:00:00 PM
ClinGen_recurrent_CNV_V1.0-hg19.bed	12.5 kB	10/8/18, 7:00:00 PM
ClinGen_recurrent_CNV_V1.0-hg38.aed	21.3 kB	4/2/19, 7:00:00 PM
ClinGen_recurrent_CNV_V1.0-hg38.bed	12.2 kB	4/2/19, 7:00:00 PM
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ClinGen_region_curation_list_GRCh38.tsv	15.5 kB	1/6/20, 2:00:00 AM
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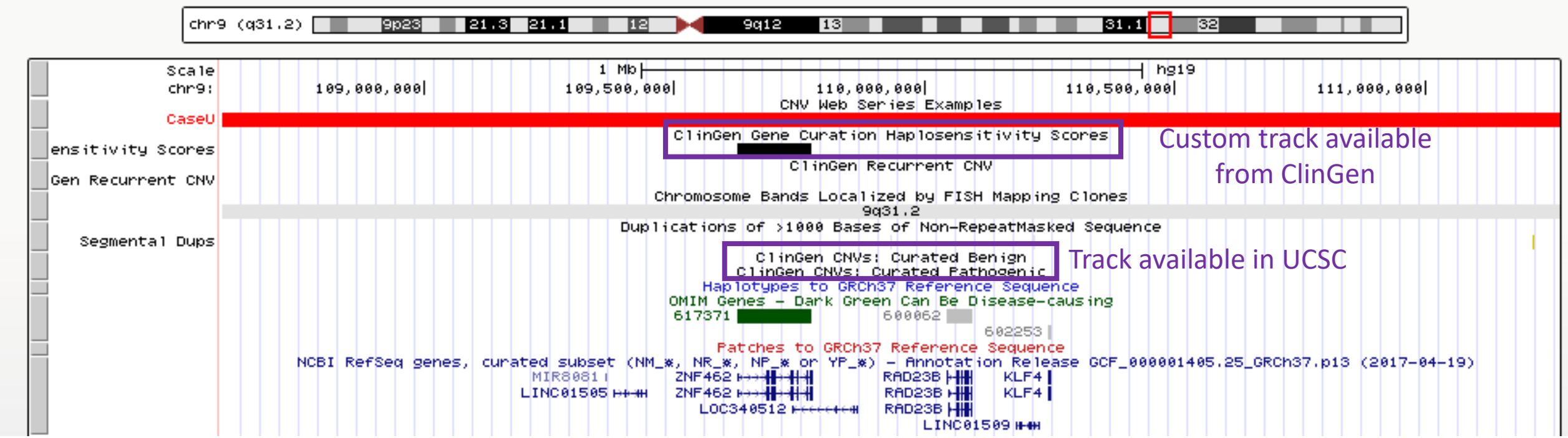
ClinGen_triplosensitivity_gene_GRCh37.bed	47.5 kB	1/6/20, 2:00:00 AM
ClinGen_triplosensitivity_gene_GRCh38.bed	47.5 kB	1/6/20, 2:00:00 AM
README	7.8 kB	4/1/19, 7:00:00 PM
all_clinical_significance/		4/10/17, 7:00:00 PM

ZNF462 appears in the ClinGen track but not the UCSC track – what does this mean?



- This means that *ZNF462* was evaluated by Dosage, but did not receive a score of 3 or Dosage Sensitivity Unlikely
- Go to the Dosage site for additional information

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

ClinGen
Clinical Genome Resource

ClinGen Dosage Sensitivity Curation Page

ZNF462

Curation Status: Complete

id: ISCA-18506

Date last evaluated: 2018-07-26

Issue Type: ClinGen Gene Curation

Gene type: protein-coding

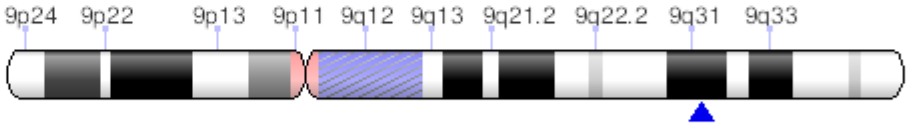
Entrez Gene: https://www.ncbi.nlm.nih.gov/gene/58499

OMIM: https://omim.org/entry/617371

ClinGen Haploinsufficiency Score: 2

ClinGen Triplosensitivity Score: 0

ExAC pLI score: 1.0



Location Information

9q31.2

GRCh37/hg19 chr9: 109,625,378-109,775,915

View: NCBI | Ensembl | UCSC

GRCh38/hg38 chr9: 106,860,154-107,013,634

View: NCBI | Ensembl | UCSC

Links

- ClinGen Curation Home Page
- ClinGen Home Page
- Help with this site
- FAQ
- Contact Us
- Pathogenic regions (nstd45)
- Curation of the ACMG 59 Genes
- FTP
- Report information on a gene

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

Haploinsufficiency score: 2
Strength of Evidence (disclaimer): Some evidence for dosage pathogenicity

Date last evaluated: July 26, 2018

Evidence for haploinsufficiency phenotype

PubMed ID	Description
28513610	Weiss et al (2017) identified predicted loss of function (LOF) variants in ZNF462 in eight patients from six families with ptosis, metopic ridging, craniosynostosis, dysgenesis of the corpus callosum, and developmental delay using whole-exome sequencing. In five families predicted LOF variants were de novo. In a family, the proband and her elder sister, with a paternally inherited nonsense variant, had normal growth and development. Her physical features included a pointed forehead (metopic ridging), bilateral ptosis, arched eyebrows, synophrys, a small upturned nose, long philtrum, and a thin upper lip. Her sister had pointed forehead, ptosis, down-slanted palpebral fissures, and a congenital capillary malformation (port wine stain) involving the right side of her neck, shoulder, and upper left arm. The father inherited the nonsense variant from his mother, and had a pointed forehead and mild left ptosis, but no other dysmorphic features. The paternal grandmother had bilateral ptosis, without metopic ridging or dysmorphic features. The paternal great grandfather was not examined but was reported to have a pointed forehead. Shared features include metopic ridging or lambdoid craniosynostosis (5/8), dysgenesis of the corpus callosum (3/8), ptosis (7/8), and developmental delay with or without autistic features (4/8).The authors proposed that ZNF462 plays an important role in embryonic development, and is associated with craniofacial and neurodevelopmental abnormalities.
29427787	Cosemans et al (2018) reported a de novo balanced translocation, t(9; 13)(q31.2; q22.1), in a patient with intellectual disability and autism spectrum disorder, metopic craniosynostosis, corpus callosum dysgenesis and dysmorphic facial features, most notably ptosis. The translocation breakpoints directly affected the genes KLF12 (chromosome 13) and ZNF462 (chromosome 9). Breakpoint mapping performed by targeted locus amplification (TLA) and sequencing showed that ZNF462 was disrupted by multiple breakpoints, resulting in the loss of three fragments and a rearrangement of the remaining fragments. Further, there was no evidence for an association of KLF12 to the phenotypic features. Therefore, haploinsufficiency of ZNF462 was responsible for the clinical features.

Haploinsufficiency phenotype comments:

De novo loss of function sequence variants have been detected in patients with craniofacial anomalies, corpus callosum dysgenesis, ptosis, and developmental delay (PMID: 28513610). In addition, a de novo balanced translocation disrupting ZNF462 in a patient with syndromic intellectual disability and autism spectrum disorder. The clinical features included metopic craniosynostosis, a corpus callosum dysgenesis and dysmorphic facial features, most notably ptosis. The translocation case is noted but not counted as evidence here as there were no functional studies proving that KLF12 was not disrupted. Due to the variable phenotype noted in the probands reported in Weiss et al. (not all probands had neurodevelopmental disorders or dysmorphic features), we are opting to give this gene a haploinsufficiency score of 2 at this time.

What does this mean for our case?

- The CNV does not overlap any <u>known</u> dosage sensitive or benign genes/genomic regions.
- Are there any predicted HI genes in the region (category 2H)?

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

Search results for 'position:9:108597937-111269478' (Refine Search)

Open-access patients 35 | CNV Syndromes 0 | DDD Research Variants 2 | Genes 15

Results | Browser

Genes: 1 to 3 of 3 (out of 15 total)

Show: OMIM Morbid DDG2P Protein coding | Filter...

Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
KLF4	9 110247133 110252763	Kruppel like factor 4	✔	-	-	1.80	0.98	View
RAD23B	9 110045418 110094475	RAD23 homolog B, nucleotide excision repair protein	✔	-	-	18.17	0.97	View
ZNF462	9 109625378 109775915	zinc finger protein 462	✔	✔	P	5.96	1.00	View

10 | Previous 1 Next

Yes. Double check the pLI scores as well as the LOEUF in gnomAD to confirm (these numbers come from ExAC).

gnomAD browser

KLF4 Kruppel like factor 4

Genome build	GRCh37 / hg19
Ensembl gene ID	ENSG00000136826
Canonical transcript ID	ENST00000374672
Region	9:110247134-110252764
References	Ensembl, UCSC Browser, and more

Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	128	163	Z = -2.43 o/e = 1.27 (1.12 - 1.45)
Missense	279.7	275	Z = 0.1 o/e = 0.98 (0.89 - 1.09)
pLoF	17.2	0	pLI = 1 o/e = 0 (0 - 0.17)

(Note updated pLI score)

gnomAD browser

ZNF462 zinc finger protein 462

Genome build	GRCh37 / hg19
Ensembl gene ID	ENSG00000148143
Canonical transcript ID	ENST00000277225
Region	9:109625379-109775916
References	Ensembl, UCSC Browser, and more

Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	558.9	601	Z = -1.4 o/e = 1.08 (1 - 1.15)
Missense	1412.4	1058	Z = 3.35 o/e = 0.75 (0.71 - 0.79)
pLoF	91.2	3	pLI = 1 o/e = 0.03 (0.01 - 0.09)

Since there are 2 predicted HI genes in the interval, should we score category 2H twice?

Evidence Type	Evidence	Suggested Points/Case	Max Score
Haploinsufficiency Predictors	**2H.** Two or more HI predictors suggest that AT LEAST ONE gene in the interval is haploinsufficient (HI)	0.15	0.15

- NO!
- 0.15 points is the maximum that can be awarded in this category

Total: 0.15 points

Section 3: Evaluation of Gene Number

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

Search results for 'position:9:108597937-111269478' (Refine Search)

Open-access patients 35 | CNV Syndromes 0 | DDD Research Variants 2 | Genes 15

Results | Browser

Genes: 1 to 3 of 3 (out of 15 total)

Show: OMIM Morbid DDG2P Protein coding

Filter...

Name	Location	Description	OMIM	Morbid	DDG2P	%HI	pLI	Links
KLF4	9 110247133 110252763	Kruppel like factor 4	✔	-	-	1.80	0.98	View
RAD23B	9 110045418 110094475	RAD23 homolog B, nucleotide excision repair protein	✔	-	-	18.17	0.97	View
ZNF462	9 109625378 109775915	zinc finger protein 462	✔	✔	P	5.96	1.00	View

10 | Previous | 1 | Next

- There are only 3 protein-coding genes in the interval (category 3A, 0 points).

Total: 0.15 points

Section 4: Detailed Evaluation of Genomic Content

- Where to start?
  - *KLF4, RAD23B,* or *ZNF462*?
- *ZNF462* has already been evaluated by ClinGen Dosage – this is a logical first place to start
  - Use evidence already documented there, then search literature for new information since date last evaluated.
  - Can we accumulate enough evidence to say that *ZNF462* is haploinsufficient?

ZNF462 Evidence Evaluation: Weiss *et al.* 2017

- Describe probands from 6 families (8 total individuals) with predicted LOF variants in *ZNF462*
- Per the authors: “Shared features include metopic ridging or lambdoid craniosynostosis (5/8), dysgenesis of the corpus callosum (3/8), ptosis (7/8), and developmental delay with or without autistic features (4/8). In addition, we identified overlapping dysmorphic features in most subjects such as arched eyebrows, down slanting palpebral fissures, epicanthal folds, wide philtrum, and a short upturned nose with a bulbous tip.”

PMID: 28513610

Weiss *et al.* 2017

Proband/Family	Variant	Method of Detection	Key Features	Other variants?	Comments
Family 1 (proband, sister, father, pat GM; variable expressivity)	c.3787C>T (p.Arg1263*)	WES	Metopic ridge, ptosis, +/- dysmorphic features, normal development	"No rare variants in genes previously associated with craniosynostosis or ACC."	
Proband 2	c.2979_2980delinsA (p.Val994Trpfs*147) *de novo*	Trio WES	Metopic ridge, ptosis, dysmorphic features; ASD	None reported.	
Proband 3	c.4263delA p.(Glu1422Serfs*6) *de novo*	WES	Lambdoid synostosis/metopic ridge; hypotonia; ptosis; dysmorphic features; transposition of the great arteries; developmental delay	Pat VUS in *FOXP2* (c.776-5T>G, NM_014491.3); mito VUS (m.14787T>C p.(I14T), NC_012920.1) in *MT-CYB* at 30% heteroplasmy	Consider not counting. Effects of other variants cannot be ruled out.
Proband 4	Chr9:108940763-110561397x 1(hg19) *de novo*	CMA	Hypotonia; dysgenesis of the corpus callosum; ptosis; dysmorphic features; normal development	Includes *RAD23B* and *KLF4*	Consider how similar this CNV is to the CNV under evaluation
Proband 5	Chr9:108464368-110362345 x1 (hg19) *de novo*	CMA	Mild ID; ASD; ADD; OCD; hx of ventricular septal defect; no evidence of craniosynostosis; no metopic ridge; no ptosis	Includes *TMEM38B*, *RAD23B* and *KLF4;* pat inherited 374 kb dup at 6q22.31 (classified as VUS)	Consider how similar this CNV is to the CNV under evaluation, possible contribution of other CNV
Proband 6	c.5145delC p.(Tyr1716Thrfs*28) *de novo*	Trio WES	Developmental delay; hypotonia; facial asymmetry; dysmorphic features.	None reported.	

What type of phenotype is this?

- Based on the first paper: the first 6 probands reported had a variable phenotype including a number of nonspecific features (ID, dev delay, ASD, hypotonia) and some slightly more specific (but not unique) features (ptosis, metopic ridge)
  - Unclear if this is a consistent but variable constellation of findings, or unrelated
  - Additional cases may help clarify
- Conservative approach:
  - 1 3-segregation family
  - 2 *de novo* LOF variants, parental relationships confirmed (trio-based WES)

Cosemans *et al.* 2018

- *De novo* balanced translocation (t(9; 13)(q31.2; q22.1)) in a patient with ID, ASD, metopic craniosynostosis, dysgenesis of the corpus callosum, and ptosis
- Translocation breakpoints were mapped to *KLF12* on chr13 and *ZNF462* on chr9
  - HI of *ZNF462* was assumed (due to previous clinical reports) but not functionally demonstrated
  - No functional studies of *KLF12* → cannot rule out effect of this gene
- Of note, another translocation case was reported by Talisetti *et al.* in 2003; features overlap with those reported here plus those associated with the other gene involved
- While compelling, this type of evidence should not scored
  - These and the unused cases from Weiss et al. could be used as an argument for upgrading if on the border between 2 classifications at the end.

PMID: 29427787

New! Kruszka *et al.* 2019

- Describes 14 additional individuals with LOF variants in *ZNF462*
  - Total of 24 individuals including those from Weiss and the translocation cases
- Not available at time of original Dosage Sensitivity evaluation
- Sheds additional light on phenotypic spectrum:
  - Developmental delay: 79%
  - ASD: 33%
  - Ptosis: 83%
  - Down-slanting palpebral fissures 58%
  - Metopic ridging or craniosynostosis: ~33%
  - Dysgenesis of the corpus callosum: ~25%
  - Structural heart defects: 21%

PMID: 31361404

Summary of variants in Kruszka *et al.*

- 13/14 detected by exome sequencing; 1/14 by genome sequencing
  - All putative LOF
  - 10 *de novo*; no comment on confirmation of parental relationships (trio-based WES vs. WES on proband with Sanger confirmation in parents)
  - 2 unknown inheritance
  - 1 paternally inherited with + paternal family hx (father with ptosis requiring surgery)
  - 1 maternally inherited, mosaic

Putting everything together…

- Even if we were being extremely conservative…
  - …and counting this as a non-specific phenotype
  - …and assuming parental relationships were not confirmed if not explicitly stated
  - …and not using cases where other variants were identified/effects of other genes were not ruled out
  - …we'd still have:
    - Category 4C: *De novo* LOF variant, non-specific phenotype
      - Parental relationships confirmed – 2 cases (Weiss) → 0.15 x 2 = 0.30
      - Parental relationships assumed – 10 cases (Kruszka) → 0.10 x 10 = 1.0
    - Category 4F: 4 total observed segregations (1 family in Weiss, 1 family in Kruszka)→ 0.15 points
- We have plenty of evidence to suggest that *ZNF462* is a HI gene, and that our CNV as a whole should be classified as Pathogenic

Total: >1.0 points

But *ZNF462* only has a ClinGen Dosage HI score of 2!

- New evidence emerges all the time
  - Outcomes of Dosage evaluations can change over time (upgrade or downgrade)
  - Always check the website for the most current information
  - Always check the date last evaluated
- In this case, new supportive evidence came out after the date last evaluated.
- If you come across a scenario like this in your clinical practice, please report it!
- This record will be updated to reflect this new information after this presentation.

ClinGen Clinical Genome Resource

ClinGen Dosage Sensitivity Curation Page

Links

- ClinGen Curation Home Page
- ClinGen Home Page
- Help with this site
- FAQ
- Contact Us
- Pathogenic regions (nstd45)
- Curation of the ACMG 59 Genes
- FTP
- Report information on a gene

ZNF462

Curation Status: Complete

id: ISCA-18506

Date last evaluated: 2018-07-26

Issue Type: ClinGen Gene Curation

Gene type: protein-coding

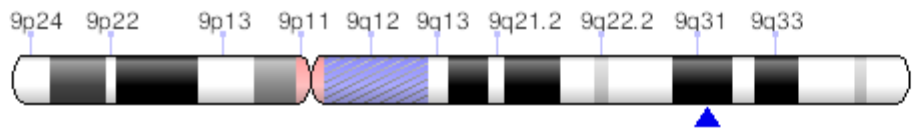
Entrez Gene: https://www.ncbi.nlm.nih.gov/gene/58499

OMIM: https://omim.org/entry/617371

ClinGen Haploinsufficiency Score: 2

ClinGen Triplosensitivity Score: 0

ExAC pLI score: 1.0



Location Information

9q31.2

GRCh37/hg19 chr9: 109,625,378-109,775,915

View: NCBI | Ensembl | UCSC

GRCh38/hg38 chr9: 106,860,154-107,013,634

View: NCBI | Ensembl | UCSC

Provide Curation Information for a Gene

ZNF462

Complete

ISCA-18506

GRCh37/hg19: chr9: 109,625,378-109,775,915

Enter Your Information

User Information

User email: *We will need an email from you in order to contact you with any questions.*

Name: *Enter you name as you would like it displayed for attribution.*

Provide any general comments you would like to add:

Dosage Sensitivity Evidence: Paper 1

Select the value for which this evidence is related: For Haploinsufficiency Against Haploinsufficiency For Triplosensitivity Against Triplosensitivity

PubMed ID 1 *Enter the PubMed ID (e.g. PMID:21844811) that provides evidence for or against dosage sensitivity.*

PubMed ID 1 Description

Please provide a brief description of why this paper provides evidence.

Population Data Check

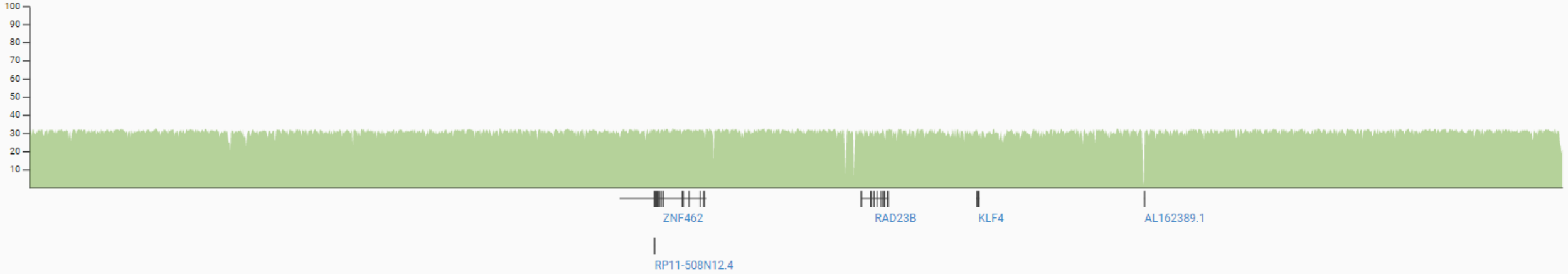
9-108597937-111269478 Change

Dataset gnomAD v2.1.1 gnomAD SVs v2.1

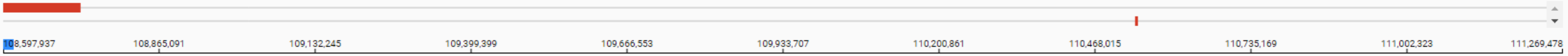
Genome build GRCh37 / hg19
Region size 2,671,542 BP
References UCSC Browser

Zoom in 1.5x 3x 10x Zoom out 1.5x 3x 10x

genome Metric: Mean Save plot



Color variants by Consequence Class

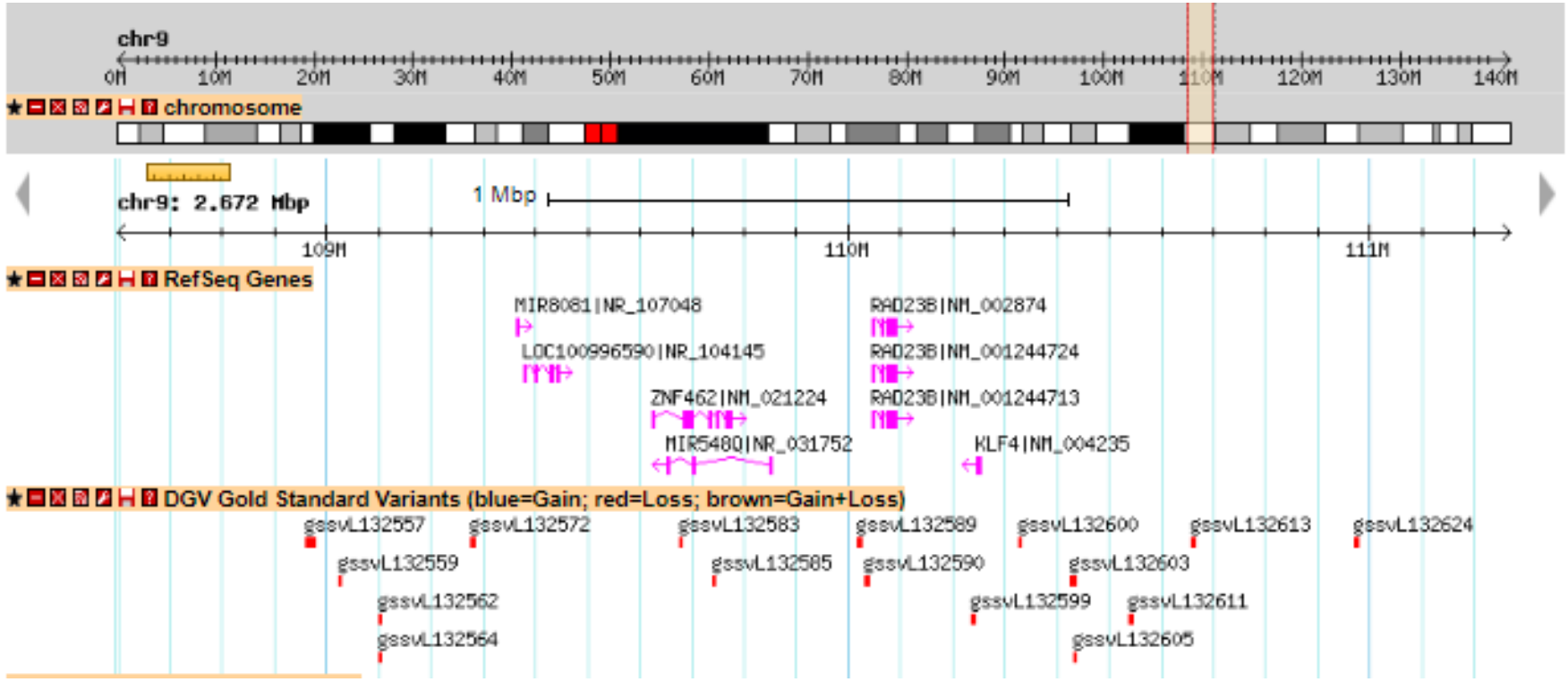


Consequences

pLoF only | Int. exon duplication only | Copy gain only | Other only

Variant ID	Source	Consequence	Class	Position	Size	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
DEL_9_104715	G	loss of function	deletion	108535399 - 108730484	195 kb	4	21694	1.84e-4	0
DEL_9_104809	G	loss of function	deletion	110537535 - 110540595	3.06 kb	13764	21692	6.35e-1	3202

Population Data Check



Conclusion: No compelling population data against pathogenicity.

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

- In this case, we already have enough compelling evidence to call this CNV Pathogenic.
- Inheritance information is unavailable, but the patient's phenotype is non-specific (developmental delay, unspecified dysmorphic features, history of VSD), but is consistent with what has been described in similar cases (Category 5G).
- What if our patient's phenotype was something seemingly unrelated, such as early infantile epileptic encephalopathy (EIEE)?
  - This CNV is still Pathogenic
  - May represent an incidental finding, provide an explanation for a phenotype not reported to you, EIEE phenotype could be masking the neurodevelopmental phenotype expected here, etc.
  - Additional testing may be warranted to elucidate a cause for the EIEE

Conclusion

- Final points based on publicly available evidence: >>1.0
- Classification: Pathogenic
  - In our case, this variant appears to be causative of the patient's presenting phenotype.
  - Even if it wasn't, there is substantial evidence that loss of *ZNF462* results in a constellation of features including variable neurodevelopmental disorders, dysmorphic features, metopic ridging/craniosynostosis, and ptosis
    - CNV should be classified as pathogenic, and clinical significance for patient under study explained in the report (e.g., causative, incidental finding, etc.)
- Points to remember:
  - Always check dates on Dosage Sensitivity evaluations; if new evidence is available that may change the score, report it to ClinGen
  - As additional evidence emerges, phenotype category (and subsequent scoring strategy) may become more clear.

Today’s Attendance URL and QR code:

https://tinyurl.com/AttendanceJan16