



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

# Questions and Answers

Facilitated by:

The ACMG/ClinGen Copy Number Variant Interpretation Working Group

# Reminder: Questions

- We do have some questions prepared that we have received in advance to review today.
- Feel free to still type your questions into the “Q&A” box
- We will answer as many as possible!

Today's Attendance URL and QR code:



<https://tinyurl.com/AttendanceMar12>

# But first...some general announcements

- You will be receiving a feedback survey via email following this webinar
  - Survey will go out to everyone who registered for the series – if you have been attending but never registered, please do so!
    - Registration: ***<https://tinyurl.com/CNVRegistration>***
  - Will collect your feedback about current series AS WELL AS your opinions about future educational initiatives
  - Survey will be open through March 31

# General Announcements (cont'd)

- Post-series CNV evaluations
  - For those of you that signed up for the pre- and post-series CNV evaluation project (back in December): you will receive your post-series CNV assignments shortly (via email)
  - Same procedure: evaluate your 5 CNVs and document results in SurveyMonkey
  - Complete as many as you can by the due date: **April 9, 2020**

# General Announcements (cont'd)

- NEW! Optional post-series CNV evaluation assignments will be available to anyone who is interested
  - You have the option of participating in this even if you were not able to sign up for the official pre-/post-series project
  - These extra post-series evaluations will be used when analyzing the effectiveness of this series.
  - Note: the only benefit to you for participating in this optional effort is extra practice. You will not receive the recognition that those participating in the official project will in any future presentations or publication of this data.
- Sign up here: <https://www.surveymonkey.com/r/PostSeriesEval>
  - Sign up will close on March 20, and CNVs will be sent out that day
  - You will receive 5 CNVs to review by April 17
- All “answers” will be posted on the CNV example page after April 17
  - Can check your work!

Question 1: If you scored evidence in category 2, but it did not reach Pathogenic, should you ALSO score evidence in Section 4?

**Section 4: Detailed evaluation of genomic content using cases from published literature, public databases, and/or internal lab data** *(Skip to section 5 if either your CNV overlapped with an established HI gene/region in section 2, OR there have been no reports associating either the CNV or any genes within the CNV with human phenotypes caused by loss of function [LOF] or copy-number loss)*

<p>Individual case evidence—de novo occurrences</p>	<p>Reported proband (from literature, public databases, or internal lab data) has either:</p> <ul style="list-style-type: none"> <li>• A complete deletion of or a LOF variant within gene encompassed by the observed copy-number loss OR</li> <li>• An overlapping copy-number loss similar in genomic content to the observed copy-number loss AND...</li> </ul>	<p>See categories below</p>	
	<p><b>4A.</b> ...the reported phenotype is highly specific and relatively unique to the gene or genomic region,</p>	<p>Confirmed de novo: 0.45 points each Assumed de novo: 0.30 points each (<i>range: 0.15 to 0.45</i>)</p>	<p>0.90 (total)</p>

# Answer 1: Yes!

- The text instructing you to skip to section 5 if your CNV overlapped with a known dosage sensitive gene in section 2 was intended only for those circumstances where you reached a “terminal” classification in section 2, either Pathogenic (P) or Benign (B).
  - Intended to help people that had achieved P or B in section 2 realize that they were not obligated to go through Section 4.
- If your CNV includes a known dosage sensitive gene/region, but for whatever reason you did NOT score 1 or -1, continue to accumulate points through the remaining sections
  - Example: Your CNV is an intragenic deletion of a known HI gene that is believed to be in-frame. The role of this region in protein function is unknown, and population variants in this region are rare. The variant is believed to remove less than 10% of the protein (Category 2E, default 0.30 points).
  - Look for similar cases in category 4 to see if you can accumulate enough evidence to reach P.

# Question 2: When is it appropriate to use public database cases as evidence?

- Consider the following:
  - Do the submitters provide a variant classification? If so, do they provide a rationale for their classification? If not, do I have enough information about this variant to come to my own conclusion?
  - What is the phenotype of this case? Does it match what is expected/my case? Is it specific or non-specific?
  - Can I tell the method by which this variant was identified? How confident am I that other potential causes for this phenotype have been ruled out?
  - Have any other potentially causative variants been identified in this individual?
  - Am I provided with any inheritance information? If so, am I provided with any phenotype information on the parents?
  - Do I *need* this case in order to complete my assessment? Are other, more straightforward cases available?

# Question 2: Example

- Remember case W, the duplication involving the *LMNB1* gene associated with adult-onset leukodystrophy...
- Would we count these two cases as evidence?

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

Search results for 'patientid:401274' (Refine Search)

Open-access patients 1 CNV Syndromes 0 DDD Research Variants 0 Genes 0

Results Karyotype

Search results: 1 to 1 of 1

DECIPHER ID	Phenotype(s)	Open-Access Variants	Contact
401274	Autistic behavior, Delayed speech and language development, EEG abnormality, Emotional lability, Hoarse voice, Hyperactivity, Large hands, Long foot, Myopia, Narrow mouth, Pain insensitivity, Pes planus, Protruding ear, Recurrent infections	1	

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

Search results for 'patientid:314220' (Refine Search)

Open-access patients 1 CNV Syndromes 0 DDD Research Variants 0 Genes 0

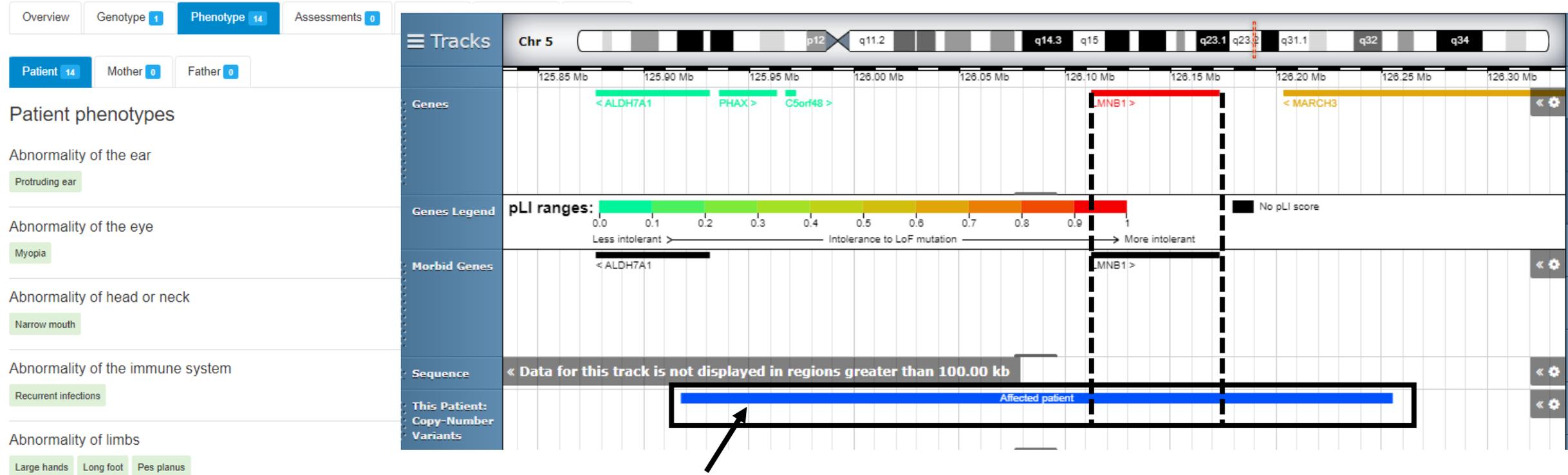
Results Karyotype

Search results: 1 to 1 of 1

DECIPHER ID	Phenotype(s)	Open-Access Variants	Contact
314220	Cognitive impairment	3	

# Patient 401274

Patient: 401274



Patient: 401274

Overview	Genotype 1	Phenotype 14	Assessments 0	Karyotype	Citations 0
Age at last clinical assessment					15
Chromosomal sex					46XY
Open-access consent					Yes

# Patient 314220

Patient: 314220

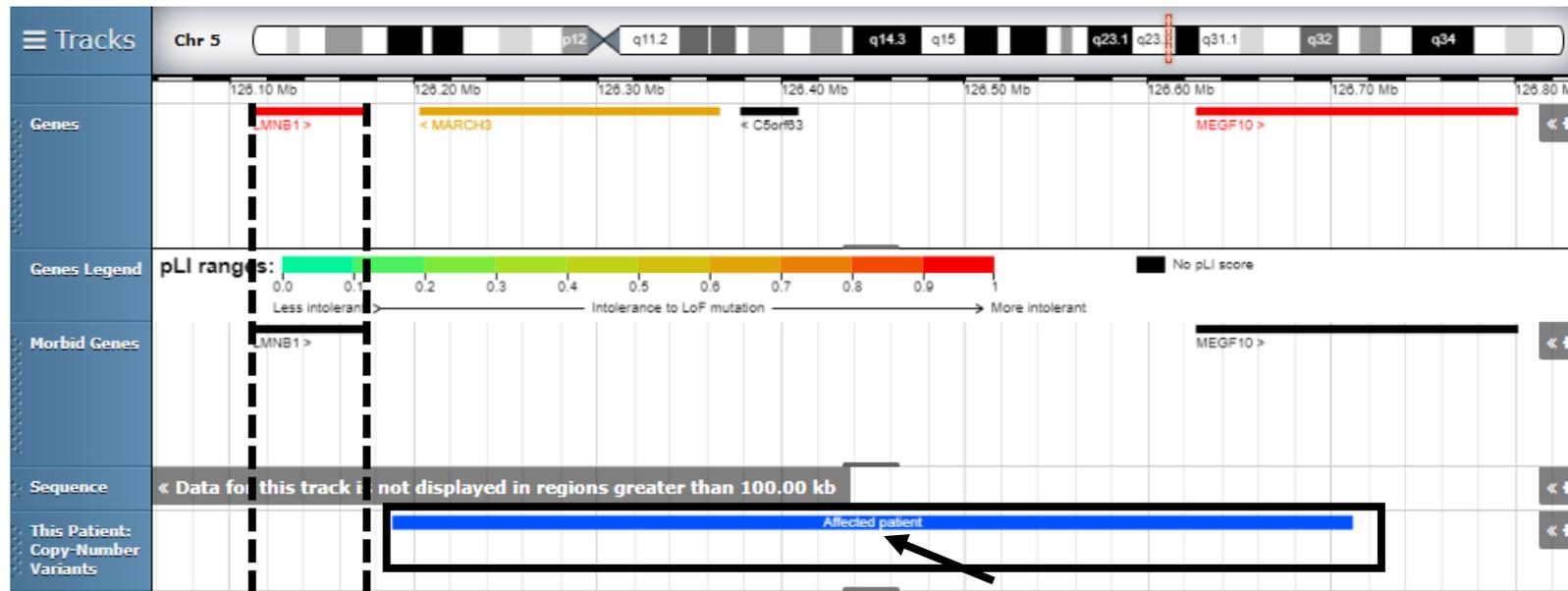
- Overview
- Genotype 3
- Phenotype 1
- Assessments 0
- Karyotype
- Citations 0
- Contacts

Age at last clinical assessment	unknown
Chromosomal sex	46XX
Open-access consent	Yes

Search results: 1 to 1 of 1

DECIPHER ID	Phenotype(s)	Open-Access Variants
314220	Cognitive impairment	3

Location	Class / Mean Ratio	Size	Genes	Inheritance / Genotype	Pathogenicity / Contribution
<a href="#">1</a> <a href="#">170868415</a> <a href="#">171480090</a>	Duplication 0.58	611.68 kb	14	Unknown Heterozygous	Uncertain Uncertain
<a href="#">5</a> <a href="#">126188676</a> <a href="#">126711285</a>	Duplication 0.58	522.59 kb	4	Unknown Heterozygous	Uncertain Uncertain
<a href="#">20</a> <a href="#">32808433</a> <a href="#">33036715</a>	Duplication 0.58	228.28 kb	5	Unknown Heterozygous	Uncertain Uncertain



# Question 3: What resources/websites would you recommend for analyzing functionally important domains or exons in a gene?

- Most often, this requires review of the literature.
  - NCBI's Entrez Gene and Ensembl – basic gene information
- Resources that can be helpful in some cases:
  - ClinGen Variant Curation Expert Panels (VCEPs)
    - Approved VCEPs will specify the mutational hot spots and/or critical/well-established functional domain as part of their specifications for the PM1 criteria
    - These are available on the ClinGen website (<https://clinicalgenome.org/working-groups/sequence-variant-interpretation/>)

<b><u>MODERATE EVIDENCE OF PATHOGENICITY</u></b>	
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.
	<b>PTEN EP Specification:</b> Defined to include residues in one of PTEN's catalytic motifs, which include the WPD loop (residues 90-94), P-loop (also described as phosphatase core, residues 123-130), and the TI-loop (residues 166-168) (NP_000305.3) (Lee 1999).

Screenshot of ClinGen  
*PTEN* VCEP's PM1  
specification  
(More detail in  
Mester *et al.* 2018,  
PMID: 30311380)

# Answer 3: Resources that can be helpful

- For transcripts: MANE
  - **M**atched **A**nnotation from the **N**CBi and **E**MBL-**E**BI
  - MANE Select: Collaboration between the two groups to identify one transcript for each protein-coding locus
    - Must match GRCh38 sequence
    - 100% identical between the RefSeq and corresponding Ensembl transcript
    - Well-supported, expressed, conserved
    - Representative of biology at each locus
  - MANE Plus: Additional well-supported transcripts of particular interest (e.g., for clinical reporting)

## Index of /refseq/MANE/MANE\_human/release\_0.8/

 [parent directory]

	Name	Size	Date Modified
	<a href="#">MANE.GRCh38.v0.8.select_ensembl_genomic.gff.gz</a>	6.3 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_ensembl_genomic.gtf.gz</a>	5.0 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_ensembl_protein.faa.gz</a>	5.7 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_ensembl_rna.fna.gz</a>	16.4 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_genomic.gff.gz</a>	5.9 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_genomic.gtf.gz</a>	5.8 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_protein.faa.gz</a>	5.1 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_protein.gpff.gz</a>	33.5 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_rna.fna.gz</a>	15.9 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.select_refseq_rna.gbff.gz</a>	60.4 MB	12/26/19, 1:27:00 PM
	<a href="#">MANE.GRCh38.v0.8.summary.txt.gz</a>	849 kB	12/26/19, 1:27:00 PM

[ftp://ftp.ncbi.nlm.nih.gov/refseq/MANE/MANE\\_human/release\\_0.8/](ftp://ftp.ncbi.nlm.nih.gov/refseq/MANE/MANE_human/release_0.8/)

# Answer 3: Resources that can be helpful

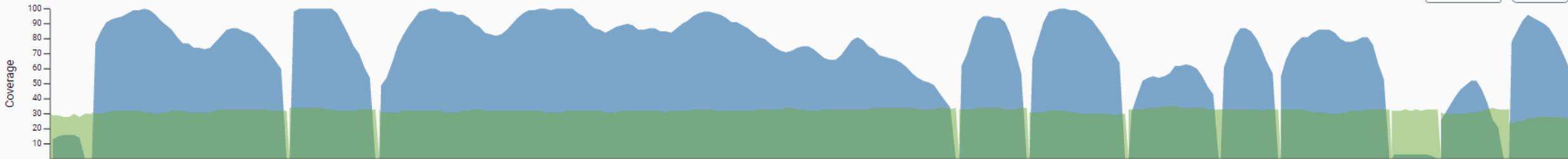
**ZEB2** zinc finger E-box binding homeobox 2

Dataset gnomAD v2.1.1 gnomAD SVs v2.1

Genome build GRCh37 / hg19  
Ensembl gene ID ENSG00000169554  
Canonical transcript ID ENST00000558170  
Region 2:145141649-145282148  
References [Ensembl](#), [UCSC Browser](#), and more

## Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	259.2	264	Z = -0.23 o/e = 1.02 (0.92 - 1.13)
Missense	663.9	378	Z = 3.94 o/e = 0.57 (0.52 - 0.62)
pLoF	44.3	1	pLI = 1 o/e = 0.02 (0.01 - 0.11)



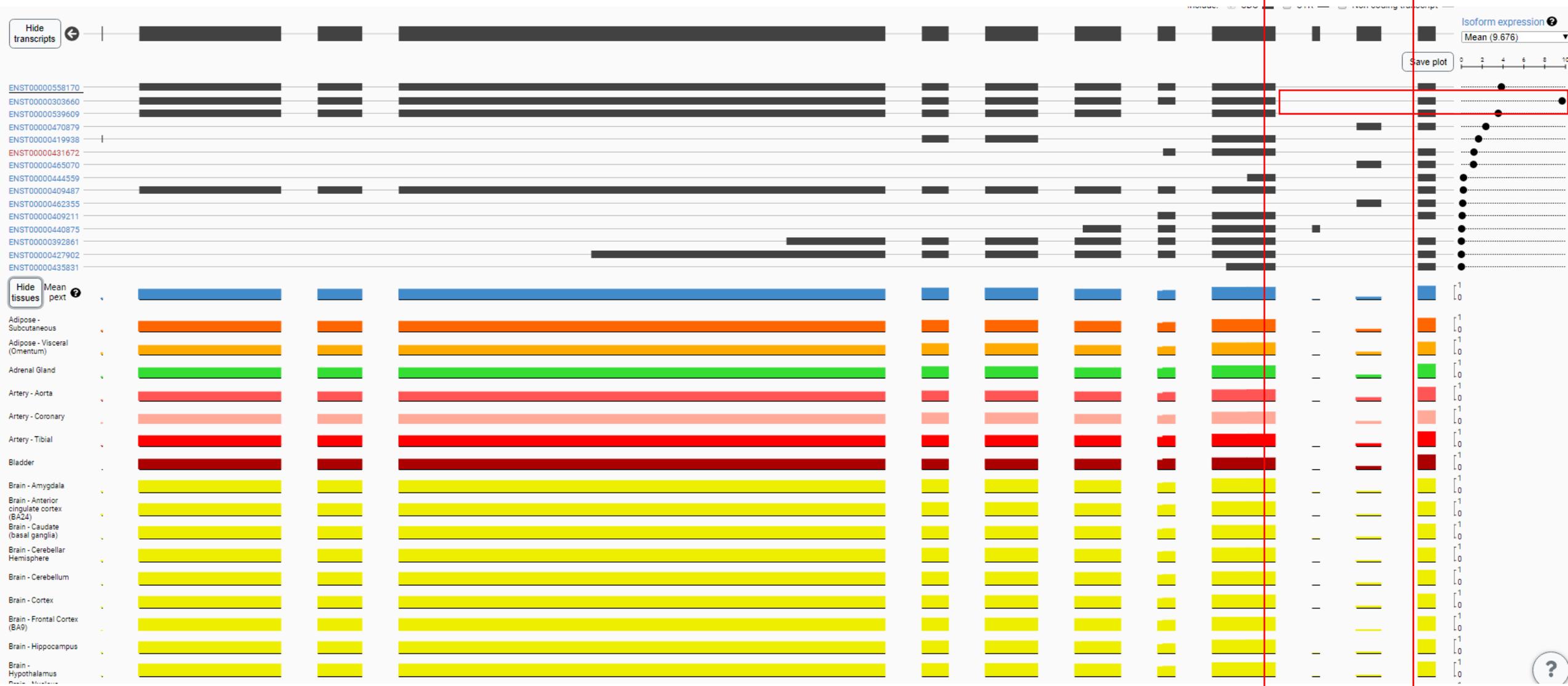
Show transcripts  
Show tissues

Include:  CDS  UTR  Non-coding transcript

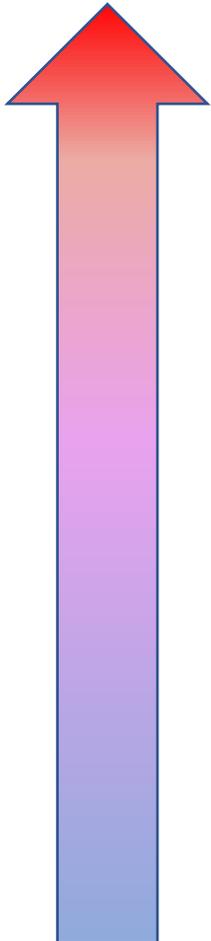
ClinVar pathogenic and likely pathogenic variants (140)

gnomAD v2.1.1 (4)

Filter to selected gnomAD variants Bins All variants



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



## Highly specific, relatively unique

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

## Highly specific, not necessarily unique

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

## Non-specific and/or high genetic heterogeneity

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

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

# Question 5: Has AI (artificial intelligence) been used to help with classification? Could this be done in the future?

- ClinGen is not aware of anyone actively using AI to help with classification.
  - Anyone in the audience?
- There are some aspects of the scoring metric that can likely be automated now:
  - Gene count
  - Overlap with ClinGen Dosage Sensitivity Curations
- Possible that AI can help with other aspects in the future
  - Will need to be carefully tested
  - Will not be able to substitute for clinical judgement and human evaluation in all circumstances
- Companies currently working on tools
  - ClinGen does not formally endorse any outside tools, but all are free to use the metrics and supporting material to create tools that will be helpful to the community.
  - Always evaluate for yourself!

# Question 6: Is there a consensus about the reporting of variants associated with adult-onset disorders in the context of prenatal diagnosis?

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**ACMG STATEMENT** | **Genetics  
inMedicine**



## The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG)

Kristin G. Monaghan, PhD<sup>1</sup>, Natalia T. Leach, PhD<sup>2</sup>, Dawn Pekarek, MD<sup>3</sup>, Priya Prasad, MD<sup>4</sup> and Nancy C. Rose, MD<sup>5</sup>; on behalf of the ACMG Professional Practice and Guidelines Committee

**Disclaimer:** This points to consider document is designed primarily as an educational resource for medical geneticists and other clinicians to help them provide quality medical services. Adherence to this points to consider document is completely voluntary and does not necessarily assure a successful medical outcome. This points to consider document should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinician should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen.

Clinicians are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this points to consider document. Clinicians also are advised to take notice of the date this points to consider document was adopted, and to consider other medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

**Keywords:** exome; prenatal diagnosis; fetal anomalies

*Genetics in Medicine* (2020) <https://doi.org/10.1038/s41436-019-0731-7>

### *Fetal incidental findings*

Incidental findings include variants in genes unrelated to the primary test indication that are not included on the ACMG secondary findings list. Incidental findings for the fetus could include clinically significant variants in genes associated with neurodevelopmental disorders, intellectual disability, or metabolic conditions that may not present with an ultrasound anomaly.

- Highly penetrant pathogenic variants detected in genes unrelated to the fetal phenotype, but known to cause moderate to severe childhood onset disorders, are recommended to be reported. Many of these disorders, especially those associated with nonsyndromic intellectual disability/neurodevelopmental disorders and metabolic conditions, are not detectable with fetal imaging.
- Regardless of ACMG variant classification, it is recommended that variants without a known fetal or childhood phenotype not be reported.
- Regardless of ACMG variant classification, it is recommended that fetal carrier status for autosomal recessive (male and female fetuses) and X-linked disorders (female fetuses) unrelated to the test indication not be reported.

PMID:31911674

# Question 7: How do I use the scoring metrics to evaluate CNVs on the X chromosome?

- In GENERAL: just as you would any other CNV!
  - Some modifications may need to be made given the unique circumstances that can be associated with CNVs on the X chromosome
- ClinGen is currently working on specific guidance in this area
- In the meantime, keep in mind the following:
  - Different possible scenarios depending on the gene(s)/disease(s) involved:
    - Males predominantly affected, females ranging from “normal” to affected (most common)
    - Females predominantly affected, male lethal
  - Use caution before awarding negative points for apparent non-segregation
  - Use clinical judgement when interpreting the results of X-inactivation studies in females.

# Using the scoring metrics for CNVs on the X chromosome (cont'd)

- Realize that you may not be able to use the *de novo* category as frequently due to the (often) inherited nature of X-linked variants
- Most often evidence will be in the form of:
  - Segregation evidence
    - Keep in mind the category maximum and when it may be appropriate to override (see Example W on the January 30 webinar or <https://clinicalgenome.org/tools/cnv-webinar/examples/>)
    - Don't artificially inflate your segregation count with genotype +, unaffected females that are NOT obligate carriers (see January 30 webinar for review of segregation counting with an X-linked pedigree)
  - Single cases of affected males with no family history, variant inherited from an unaffected mother
    - Evaluate these carefully and be conservative
    - If you are dealing with neurodevelopmental disorders (NDDs), remember how many genes associated with NDD are on the X chromosome! Are you sure yours is the correct one?
    - Have other potential causes of the phenotype been ruled out? Is there any functional data to support the idea that a given variant is the causative variant?

# Question 8: How do I use the scoring metric to evaluate AR genes?

- The scoring metrics were created for the evaluation of autosomal dominant genes/genomic regions of reasonable penetrance.
- Supplemental Material 1: When evaluating a multi-gene CNV, “give precedence to the genes in the region that are associated with dominantly inherited disorders caused by an appropriate mutational mechanism.”
- While the scoring metrics are not built to formally evaluate AR genes, the subsequent guidance on reporting is asking people not to IGNORE situations in which the patient is clearly at least a carrier for well-established conditions in which LOF is a disease mechanism.

# Using the scoring metrics for AR genes (cont'd)

- What do we mean when we say “genes associated with well-established” conditions? Some examples include...
  - ClinGen Gene-Disease Validity evaluations of Strong or Definitive
  - GeneReviews
  - Other sources demonstrating convincing evidence supporting the gene-disease relationship
- Carefully evaluate whether LOF is an established disease mechanism
- If these things are not apparent, it may not be appropriate to call the variant “Pathogenic” (based on this gene alone)
  - Consider mentioning the gene and the possibility of carrier status in the report
  - Not mandatory if this is not feasible (e.g., 100 gene deletion)

The screenshot shows the ClinGen website's 'Gene Validity Curations' page. At the top, there is a navigation bar with links for 'Get Started', 'About Us', 'Curation Activities', 'Working Groups', 'Expert Panels', 'Documents & Announcements', and 'Tools'. Below this is a search bar with the text 'Search: Gene' and 'Gene...' and a 'Go' button. A red box highlights a 'Browse Curations' button. The main heading is 'Gene Validity Curations'. Below the heading, it says 'Curation Count: 876' and a red box highlights a 'Download Summary' button. A table with a search filter 'Filter table...' is displayed. The table has columns for 'Gene', 'Disease curated', 'MOI', 'SOP', 'Classification', and 'Released'. Two rows are visible, both for the gene 'A2ML1'. The first row is for 'Noonan syndrome with multiple lentigines' (MONDO:0007893) with MOI 'AD' and SOP 'SOP5'. The second row is for 'cardiofaciocutaneous syndrome' (MONDO:0015280) with MOI 'AD' and SOP 'SOP5'. Both rows show 'No Reported Evidence' in the Classification column and '06/07/2018' in the Released column.

Gene	Disease curated	MOI	SOP	Classification	Released
A2ML1	Noonan syndrome with multiple lentigines MONDO:0007893	AD	SOP5	No Reported Evidence	06/07/2018
A2ML1	cardiofaciocutaneous syndrome MONDO:0015280	AD	SOP5	No Reported Evidence	06/07/2018

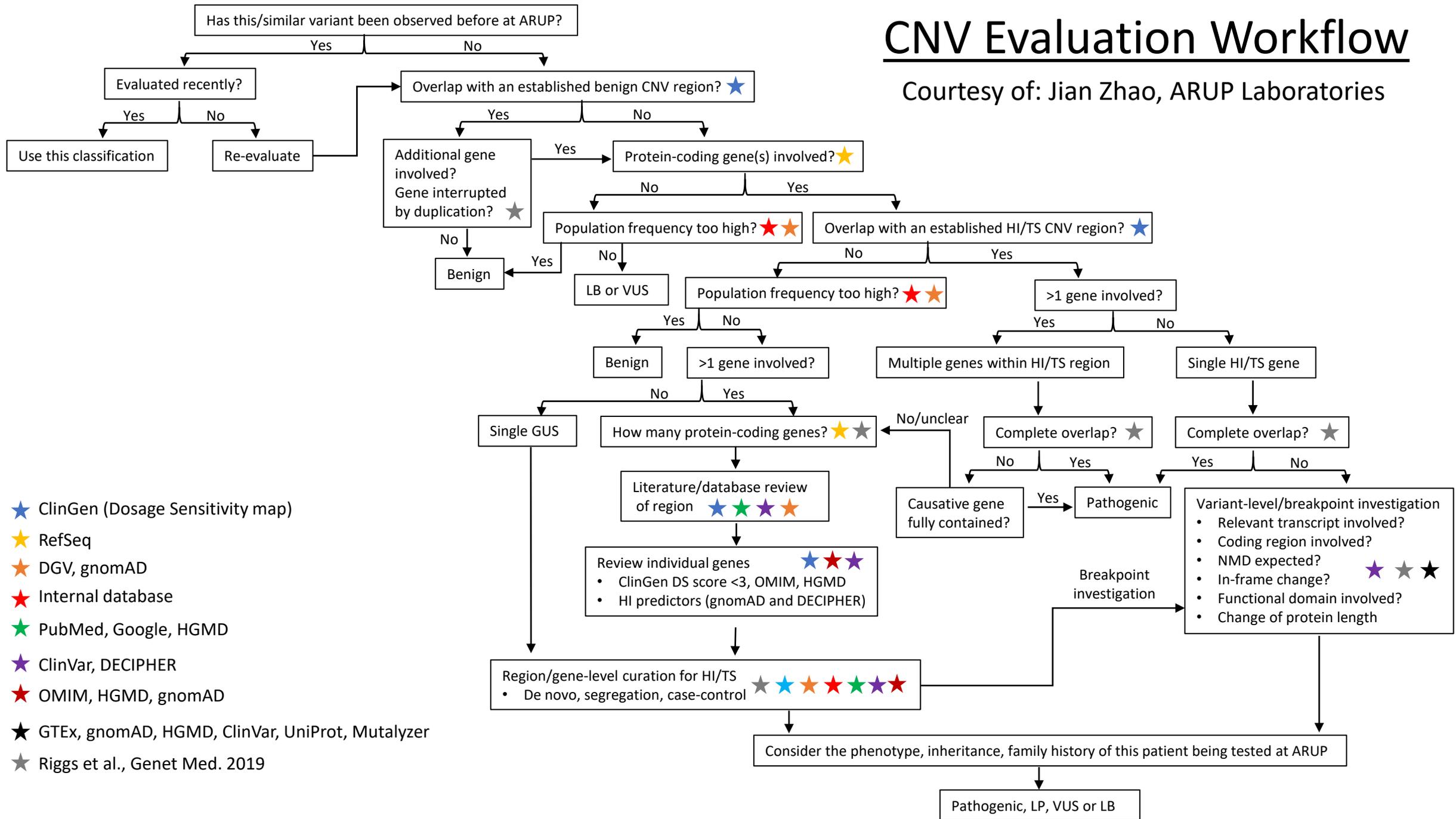
# Question 9: How do I use the scoring metrics to evaluate recurrent regions?

- The scoring metrics were created for the evaluation of autosomal dominant genes/genomic regions of reasonable penetrance.
- We recognize that several of the recurrent regions pose considerable difficulty in terms of classification and reporting due to their association with reduced penetrance/variable expressivity
  - Lack of consensus within the community
- ClinGen is currently working on specific guidance in this area:
  - Recurrent region dosage evaluation process: See January 23 webinar (Dosage Sensitivity Map)
  - ClinGen Low Penetrance/Risk Allele working group recommended terminology: See March 5 webinar (Reporting)
    - Also: <https://clinicalgenome.org/working-groups/low-penetrance-risk-allele-working-group/>
  - Additional educational/consensus-building activities being developed – stay tuned!

Question 10: How are clinical laboratories implementing the new technical standards?

# CNV Evaluation Workflow

Courtesy of: Jian Zhao, ARUP Laboratories



- ★ ClinGen (Dosage Sensitivity map)
- ★ RefSeq
- ★ DGV, gnomAD
- ★ Internal database
- ★ PubMed, Google, HGMD
- ★ ClinVar, DECIPHER
- ★ OMIM, HGMD, gnomAD
- ★ GTEx, gnomAD, HGMD, ClinVar, UniProt, Mutalyzer
- ★ Riggs et al., Genet Med. 2019

# In conclusion...

- The current ACMG/ClinGen technical standards for constitutional CNVs represent an initial step toward a more structured, transparent method of CNV evaluation
  - We anticipate that updates and changes will be required over time as we gain more experience and knowledge.
  - YOU are our partner in this process – please continue to send in suggestions, feedback, etc.
    - Recognize that changes may not happen instantaneously
    - Remember that suggestions made by ClinGen outside of the published document are not reviewed or approved by ACMG until there is a formal update
- Keep in touch with ClinGen for the latest updates on our work (CNV and beyond)
  - Twitter: @ClinGenResource
  - YouTube: ClinGen Resource
  - Indicate that you DO want to be updated in our feedback survey
    - Will add you to mailing list
  - Volunteer to curate for Dosage Sensitivity: <https://tinyurl.com/ClinGenVolunteer>

Other questions?

Today's Attendance URL and QR code:



<https://tinyurl.com/AttendanceMar12>