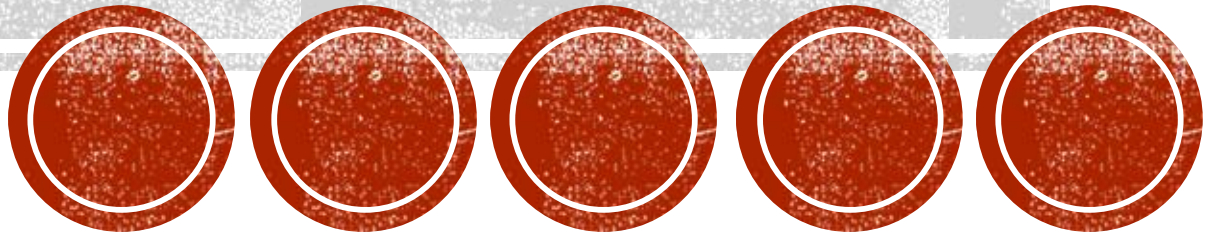
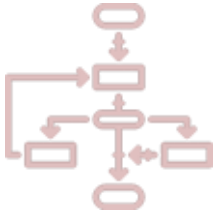
FOLLOW-UP AFTER MOLECULAR SCREENING RESULTS

Amy Gaviglio, MS, LCGC
Genetic Counselor
G2S Corporation/CDC Contractor



OUTLINE



The Testing and Notification Plan

Variant Nomenclature

Communicating Molecular Results to Primary Providers

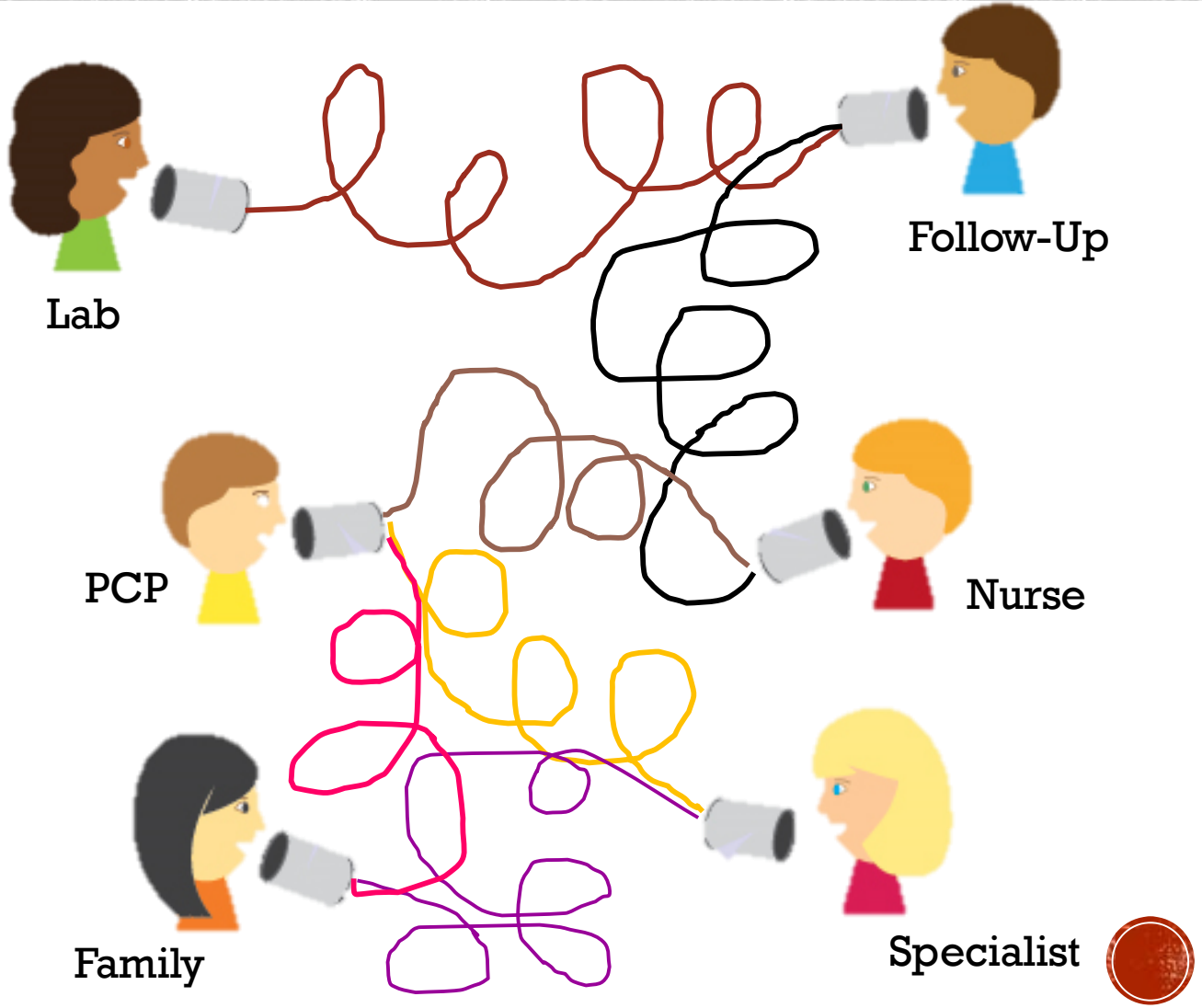
Case Studies

#1

ISSUE IN HEALTHCARE COMMUNICATION IS THE ILLUSION IT HAS TAKEN PLACE

Adapted from http://digitalpa.us

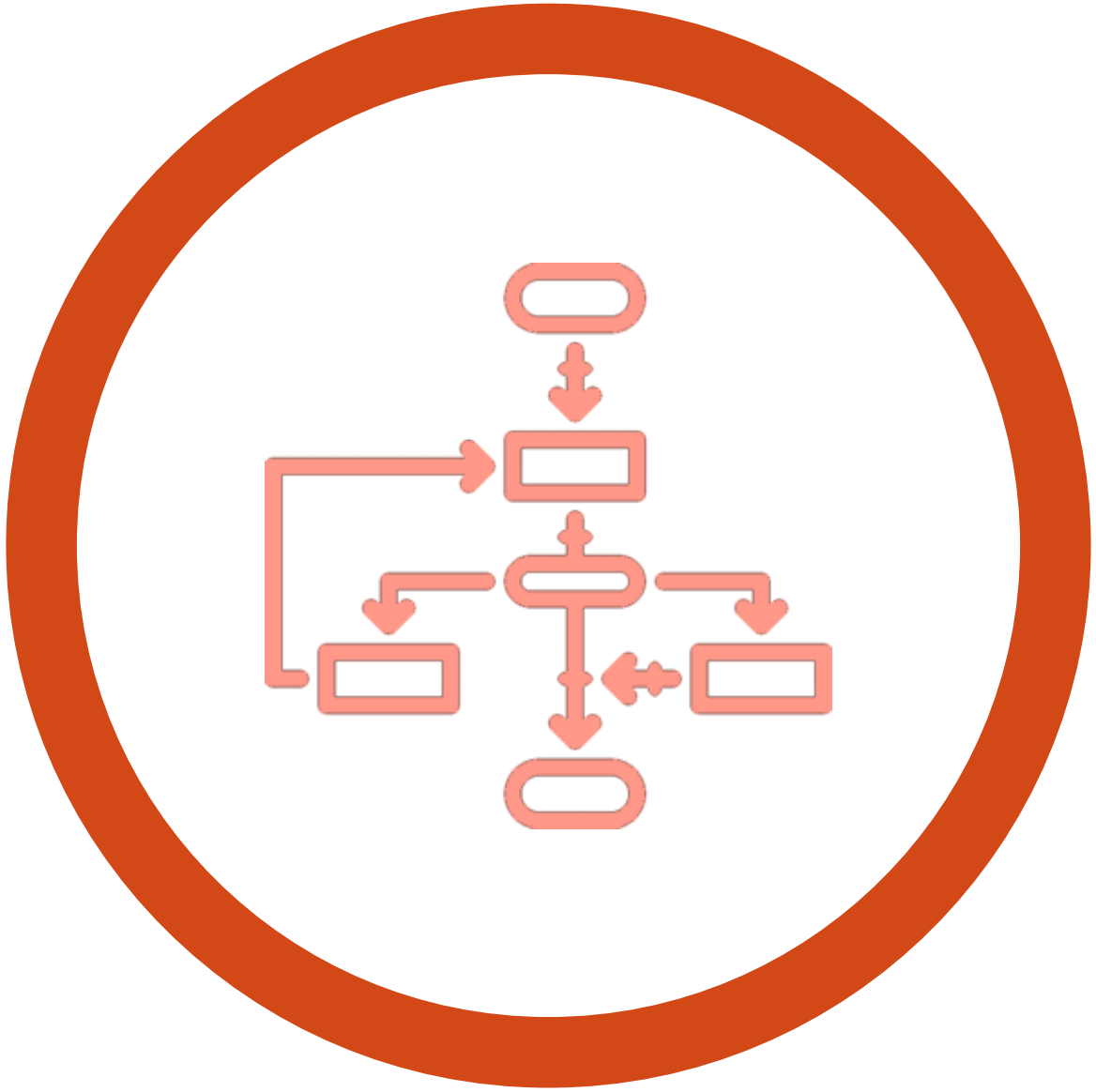
COMMUNICATION RISKS IN NEWBORN SCREENING



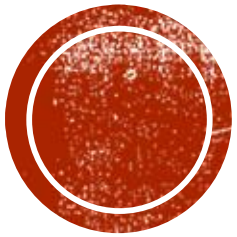
WHY IS COMMUNICATION SO IMPORTANT WITH MOLECULAR TESTING?

- **NOTE:** This is not new!
  - But… new conditions and technologies present additional educational needs
    - Late Onset Variants
    - Carrier Status
    - 2+ Variant Findings
    - Pseudodeficiency

- **These individuals are our conduits to our main stakeholders… the families**
  - Fact sheets/resources are only as good as their dissemination
  - Our screening results are only as good as the follow-up done
  - Thorough understanding of the screening process, results, and next steps improves multiple facets of follow-up



THE TESTING & NOTIFICATION PROCESS



EXTERNAL PROCESSES AND COMMUNICATION

MOLECULAR IMPLEMENTATION: WHY?

- **Discuss the why – why are you adding molecular testing?**

- **Three primary reasons:**
  - **To improve sensitivity/specificity**
    - Poor 1st tier biochemical marker and/or no 2nd tier biochemical marker/assay
  - **To aid in diagnostic/treatment decisions**
    - *GAA* genotype and CRIM status; SCID genes and targeted treatment strategies
  - **To address health equity/access to genetic testing**
    - Ensure that all patients can obtain molecular information

- **One that is not a reason:**
  - Provide diagnostic testing
    - All screening results (even molecular ones!) need to be confirmed in some way!

MOLECULAR IMPLEMENTATION: ALGORITHM

- **Discuss screening algorithm and target conditions**
  - Do specialists want/have the capacity to see cases where molecular results suggest likely pseudodeficiency and/or variants of unknown significance?

  - **If NO:**
    - What approach, if any, can be taken by the program to rule these out prior to molecular testing?
    - Will these findings still be reported out, but with no requirement for clinical follow-up?
    - Who will be available for provider and/or family questions?

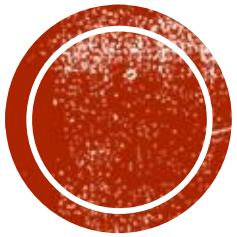
  - **If YES:**
    - What will clinical follow-up look like?
    - What is the timeline for this follow-up?

MOLECULAR IMPLEMENTATION: NOTIFICATION

- **Discuss notification strategy**
  - At what point in the screening algorithm will specialists be notified of positive results?
  - **If AFTER INITIAL BIOCHEMICAL RESULTS:**
    - What is the timeline for further tiered results?
    - Will specialists still want to see the family right away? Or only in cases above/below a certain cutoff?
    - Will a preliminary report be provided while holding the final report for molecular results?
  - **If AFTER MOLECULAR TESTING:**
    - Are specialists comfortable with the delay in turn around time?
    - Will molecular testing be run on weekends/holidays for reporting out by follow-up staff?

MOLECULAR IMPLEMENTATION: CONFIRMATION

- **Discuss whether further molecular testing will need or want to be pursued:**
  - Will specialists want to confirm the molecular results clinically? Always? Only if other testing is discrepant with molecular results?
  - For targeted variant panels, what sequencing is available clinically?
    - What is turn around time for pre-authorizations and results?
  - Be clear whether deletion/duplication testing is being done or whether this may need to be sent clinically in certain cases



INTERNAL PROCESSES AND COMMUNICATION

LIMS AND CMS CONSIDERATIONS

- **Preliminary reporting**
  - How will this be handled?
  - Can a preliminary report be created?

- **Documenting variant findings**
  - Will variant information be captured in discrete fields (ideal) or in free text?
  - Can other clinical information be captured in discrete fields to more easily tie together genotype and phenotype?

- **Where will molecular results show up on the report?**
  - Will they need to be added on to an existing interpretation as a comment or trailer or can they be built in?

CARRIER NOTIFICATION AND EDUCATION

TARGETED VARIANT PANELS	FULL SEQUENCING
• Will one variant findings still require follow-up?	• One variant findings very likely a carrier, but small residual risk may remain
• Likely to be asked residual risk of being affected by providers and families	• Will families be provided information on what it means to be carrier? By whom?

REPORT FORMAT AND LANGUAGE

- **Format**
  - What information needs to be included on reports that include molecular results and variant interpretation?
  - Will this make reports longer (yes!)?
  - How can formatting be changed to facilitate understanding of complex results?

- **Language**
  - Molecular results can be exceedingly complex
  - How will molecular results, any limitations, and recommendations be written to improve and facilitate interactions between primary care, specialists, and the patients?

JL Williams, et al., Am. J. Med Genet. 2016 May; 170A(5):1134-41
Haga et al. Genome Medicine 2014, 6:58

VARIANT NOMENCLATURE

What's in a Name?

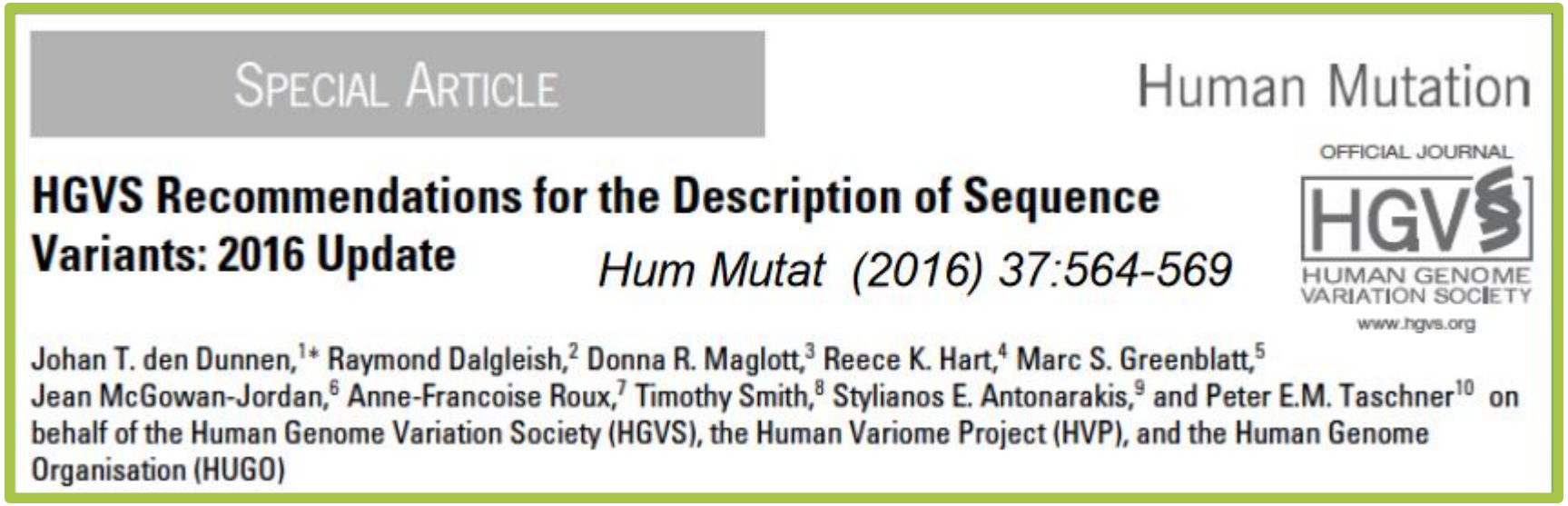
BUT WHY?

- Negative connotation with term 'mutant'
- Assumption of pathogenicity
- Consistency and allowance for re-classification

WHAT'S IN A (VARIANT) NAME?

- Standard naming (nomenclature) rules exist
  - Allows for easy and unequivocal description/communication of variants

- Dictated by the Human Genome Variation Society (HGVS)
  - http://varnomen.hgvs.org



SPECIAL ARTICLE

Human Mutation

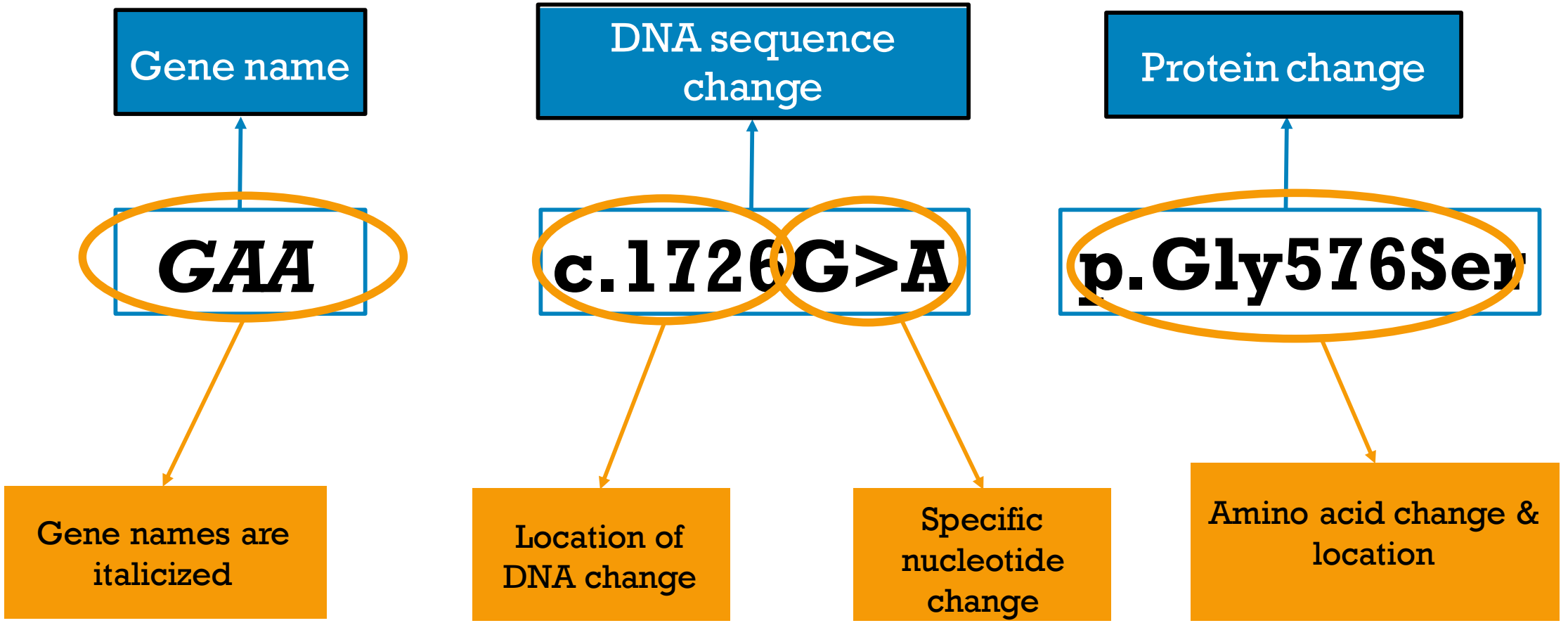
OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

HGVS Recommendations for the Description of Sequence Variants: 2016 Update

Hum Mutat (2016) 37:564-569

Johan T. den Dunnen,[1]* Raymond Dalgleish,[2] Donna R. Maglott,[3] Reece K. Hart,[4] Marc S. Greenblatt,[5] Jean McGowan-Jordan,[6] Anne-Francoise Roux,[7] Timothy Smith,[8] Stylianos E. Antonarakis,[9] and Peter E.M. Taschner[10] on behalf of the Human Genome Variation Society (HGVS), the Human Variome Project (HVP), and the Human Genome Organisation (HUGO)

VARIANT NOMENCLATURE



NBS-RELATED GENES

Condition	Gene Name(s)
Congenital Adrenal Hyperplasia	*CYP21A2*
Cystic Fibrosis	*CFTR*
Galactosemia	*GALT (GALE and GALK)*
Hemoglobinopathy	*HBA1, HBA2, and HBB*
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*
Mucopolysaccharidosis Type I	*IDUA*
Phenylketonuria	*PAH*
Pompe disease	*GAA*
Spinal Muscular Atrophy	*SMN1 and SMN2*
Very long-chain acyl-CoA dehydrogenase deficiency	*ACADVL*
X-linked adrenoleukodystrophy	*ABCD1*

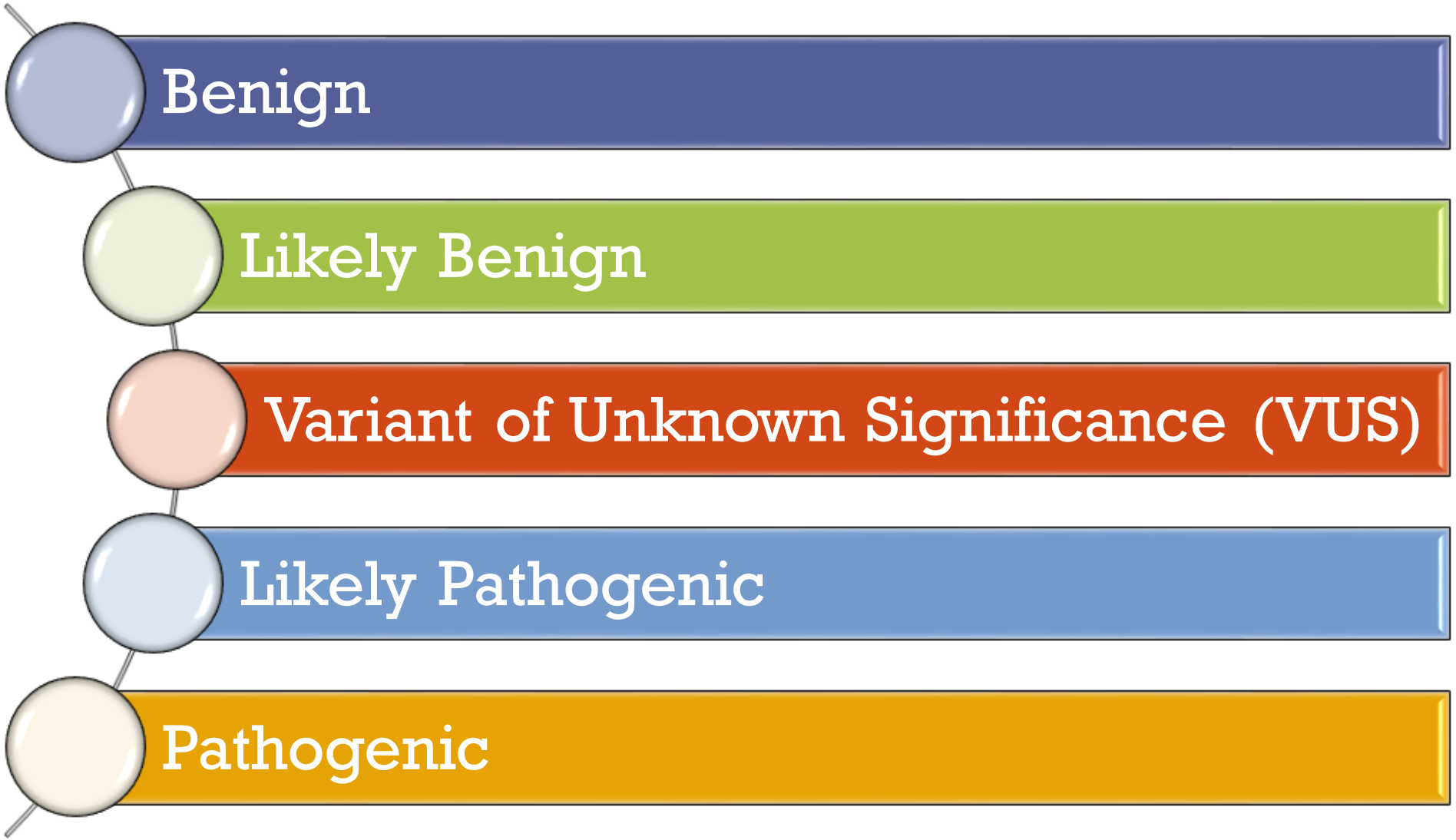
MORE VARIANT NOMENCLATURE

DNA sequence location	
c.	Coding DNA reference sequence
g.	Genomic reference sequence
Functional impact	
p.	Protein change
Nucleotide change types	
>	Substitution
Del	Deletion
Dup	Duplication
Ins	Insertion
Inv	Inversion
Fs	Frame shift
+	Change is in intron after the exon
-	Change is in intron before the exon

PRACTICE

ABCD1	c.427C>T	p.Pro143Ser
Gene for XALD	C was replaced by T at nucleotide number 427	Amino acid 143 is supposed to be proline, but is now serine
CFTR	c.1521_1523delCTT	Phe508del
Gene for Cystic Fibrosis	Nucleotides C,T, and T were deleted at nucleotide numbers 1521 to 1523	Amino acid 508 (phenylalanine) was deleted
CYP21A2	c.293-13C>G	
Gene for CAH	C was replaced by G 13 nucleotides in the intron region before nucleotide 293	Variant is in the noncoding intron region, so there is no amino acid change

ACMG VARIANT CLASSIFICATION



HOW DO VARIANTS GET CLASSIFIED?

Type of Data	Used to	Example(s)
Population databases	• Obtain the frequencies of variants in large populations • May include healthy and affected individuals	• 1000 Genomes Project • dbSNP • ExAc
Disease databases	• Primarily contact variants found in affected individuals	• ClinVar and ClinGen • Human Gene Mutation Database • CFTR2
Scientific/Medical Literature	• Determine reports of variants and associated phenotype	• PubMed • OMIM
Computational/Predictive (*in silico*)	• Determine the effect of the variant at the nucleotide and amino acid level • Help predict whether a variant damages the protein function/structure	• MutationTaster • PolyPhen-2 • SIFT

HOW DO VARIANTS GET CLASSIFIED, CONT'D?

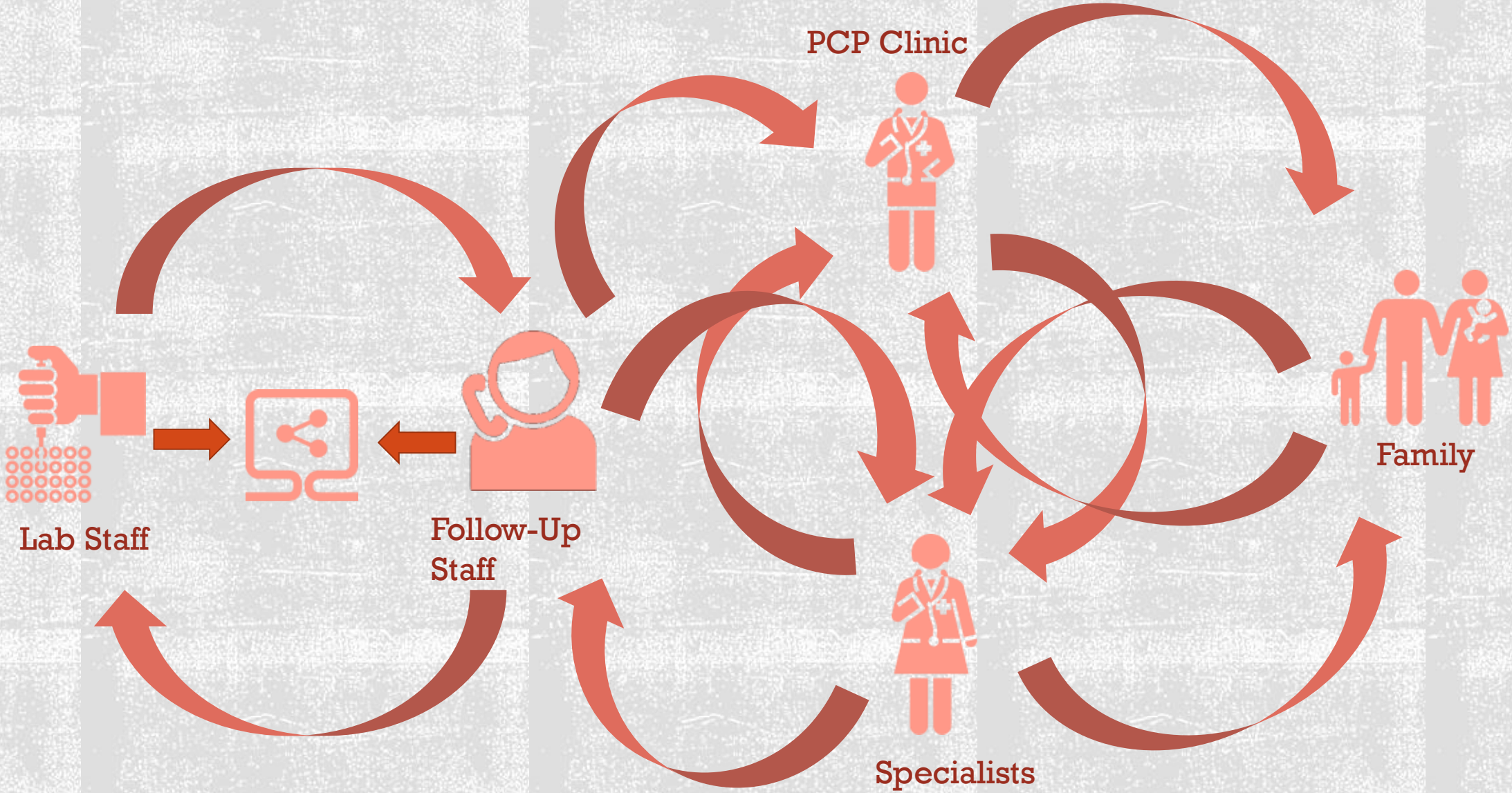
Type of Data	Used to	Example(s)
Functional Studies	• Predict an impact of a variant on protein function using enzymatic assays	• Enzyme assay on biopsied tissue
Segregation	• Determine if the variant segregates in the family with the disease	• Family history and familial genetic studies
De novo	• Determine if variant is new (e.g., not inherited) • *De novo* variants are strongly supportive of pathogenicity	• X-ALD
Allelic	• Determine whether the variant is in *cis* or *trans* with another known pathogenic variant	• Parental studies

CLINICAL USE OF VARIANT CLASSIFICATIONS

Benign	• Variant is not considered to be the cause of the disease • Testing is considered negative
Likely Benign	• Variant is not likely to be the cause of the disease • Testing may be considered negative; usually not considered clinically actionable
VUS	• Variant has characteristics of being disease-causing, but insufficient or conflicting evidence exists • Management should be based on clinical judgment; family testing may be useful
Likely Pathogenic	• Variant is considered the probable cause of the disease • Variant should be used cautiously for clinical decision-making and family risk assessment
Pathogenic	• Variant is established as disease-causing and considered the cause of the disease • Variant can be used in clinical judgement and in evaluating risk for family members

COMMUNICATING MOLECULAR RESULTS TO PRIMARY PROVIDERS

THE IDEAL COMMUNICATION SYSTEM



GENETIC LITERACY

- **Often derived from media**
  - TV
  - Movies
  - Social Media
  - News
- **Misconceptions:**
  - Timeliness
  - Absoluteness
  - Capabilities

PRIMARY CARE AND GENETIC LITERACY

- Physicians indicate an overall lack of knowledge and confidence in discussing genetic risk[1]

- Only 45% of healthcare provider respondents in one study indicated that they felt well-informed about genetic testing[2]
  - 49% felt that their genetics training had been inadequate

- Avoid "They're doctors - they know this!" mentality!

[1] Mikat-Stevens, et al., Genetics in Medicine (2015) 17, 169-176
[2] Haga, S.B., Carrig, M.M., O'Daniel, J.M. et al. J GEN INTERN MED (2011) 26: 834

COMMUNICATING MOLECULAR FINDINGS

Give Results
- Consider faxing molecular results prior to calling
- Reading variant information over the phone can be challenging

Educate
- Communicate molecular findings and variant interpretation
- Provide 'Just In Time' condition-specific information

Next Steps
- Review recommended next steps and timeline

Explore Questions
- Ask if there are any outstanding questions about the result; what to say to the family; and/or the recommended action plan

THE FUTURE

- Ever increasing need to educate and talk directly with providers
  - Need to increase understanding of sequencing technology
  - Need to increase understanding of genetic terms and implications
- Expanded role of primary care in ongoing monitoring for infants with variants of unknown significance and/or variants suggesting later onset of symptoms

CASE STUDIES

CYSTIC FIBROSIS CASE 1

- Infant is found to have a moderately elevated IRT on newborn screening
- Follow-up molecular testing using a targeted variant panel reveals the following two variants:
  - **Phe508del (F508del)** (*variant is known to be associated with classic CF*)
  - **Arg117His (R117H)** (*variant has wide phenotype – from normal to near classic CF*)
    - The "poly T" variant status affects pathogenicity
    - The child is found to have the 7T poly T variant

CYSTIC FIBROSIS CASE 1

- Sweat test comes back borderline
- Repeat sweat test also returns borderline
- Child is ultimately assigned a diagnosis of CRMS (CFTR-Related Metabolic Syndrome)

CYSTIC FIBROSIS CASE 2

- Infant is found to have very elevated IRT on newborn screening
- Follow-up molecular testing using a targeted variant panel reveals the following two variants:
  - **Phe508del (F508del)** (*variant is known to be associated with classic CF*)
  - **Glu60* (E60*)** (*variant is known to be associated with classic CF*)

CYSTIC FIBROSIS CASE 2

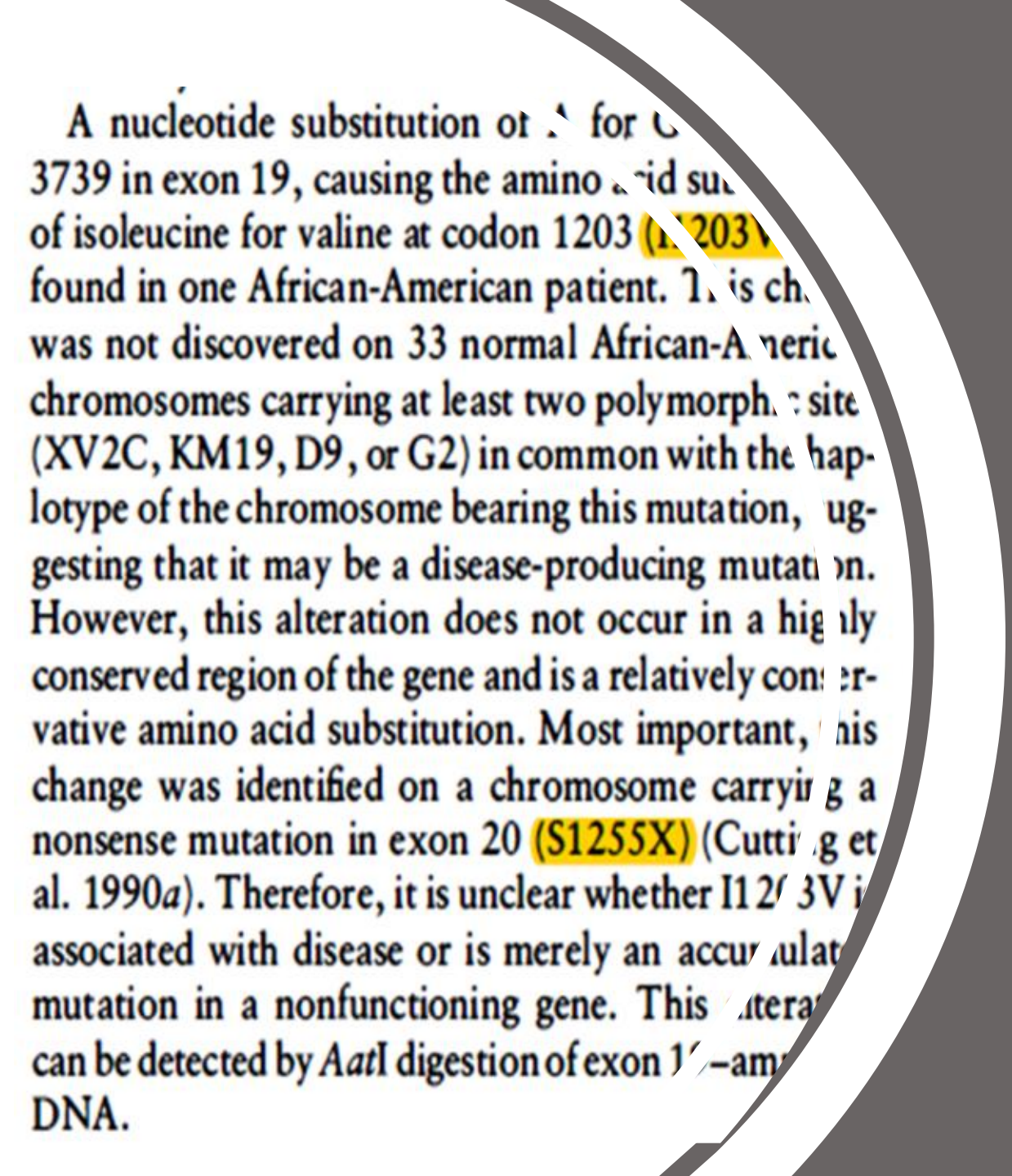
- After notification to the provider, she calls back to say that the family won't come in as they had prenatal screening and mom knows she is a carrier for the Phe508del variant, but dad was tested and his screen was negative.

CYSTIC FIBROSIS CASE 2

- Ask provider to determine what variants were tested for in the father
- Provide education on the different variant panels available and that the additional variant found is not on the common 23 variant ACMG panel

CYSTIC FIBROSIS CASE 3

- Elevated IRT (67 ng/ml) followed by DNA
  - Het for 2 variants: Ser1255* (ex. 19) and Ser1255* (ex. 20)
- CFTR2 indicates that Ser1255* (ex. 20) causes CF when found with another CF-causing variant
  - Ser1255* (ex. 19) not listed
- How should this result be handled?



A nucleotide substitution of A for G
3739 in exon 19, causing the amino acid sub
of isoleucine for valine at codon 1203 (I1203V
found in one African-American patient. This ch
was not discovered on 33 normal African-American
chromosomes carrying at least two polymorphic site
(XV2C, KM19, D9, or G2) in common with the hap-
lotype of the chromosome bearing this mutation, sug-
gesting that it may be a disease-producing mutation.
However, this alteration does not occur in a highly
conserved region of the gene and is a relatively conser-
vative amino acid substitution. Most important, this
change was identified on a chromosome carrying a
nonsense mutation in exon 20 (S1255X) (Cutting et
al. 1990*a*). Therefore, it is unclear whether I1203V is
associated with disease or is merely an accumulated
mutation in a nonfunctioning gene. This alteration
can be detected by *Aat*I digestion of exon 19–amplified
DNA.

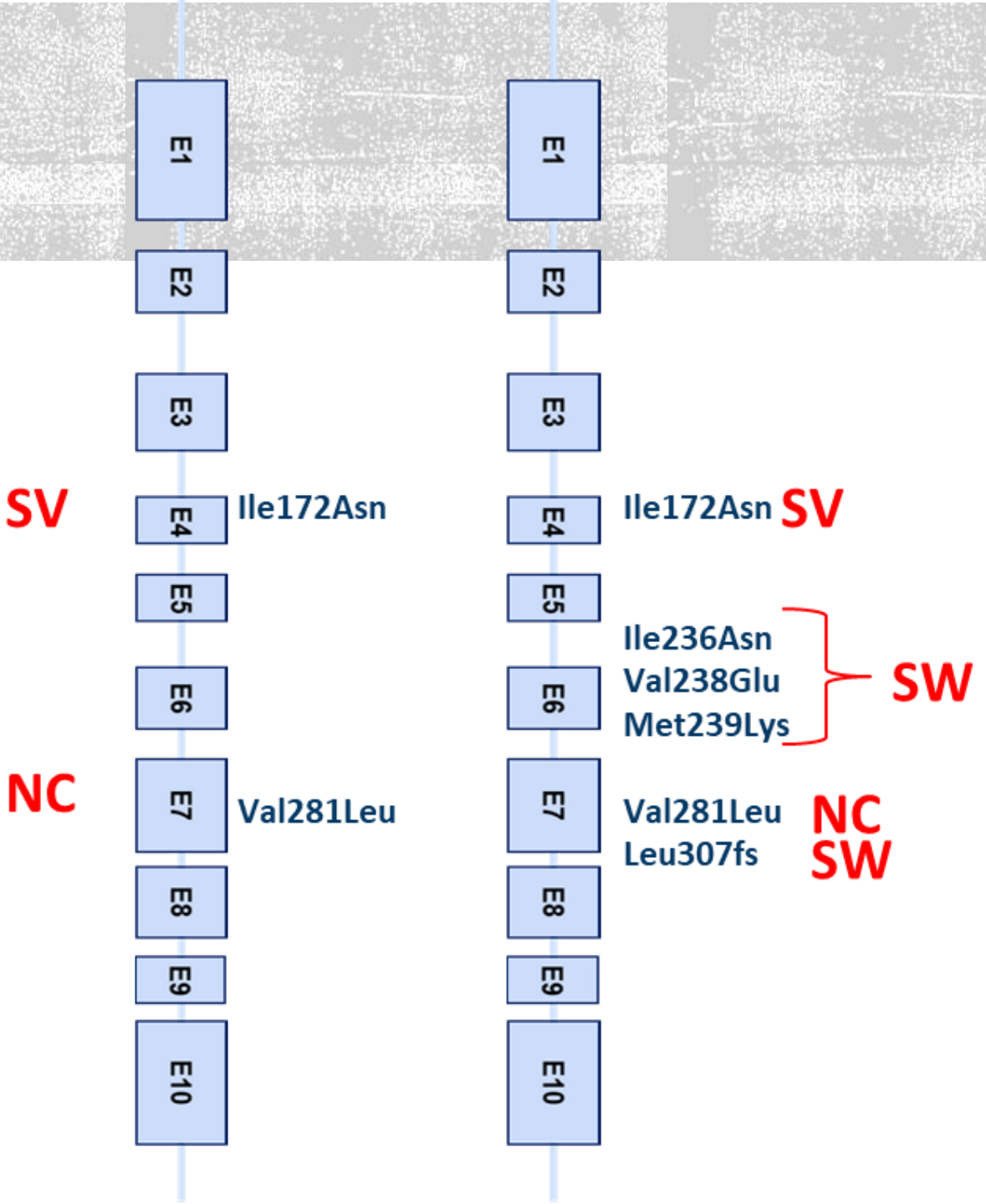
CYSTIC FIBROSIS CASE 3

- Treat as ONE variant:
  - Ser1255* (ex. 19) and Ser1255* (ex. 20) act as a haplotype
- Enzymes don't need to be started
- Sweat test results = 4 and 5 mmol/L

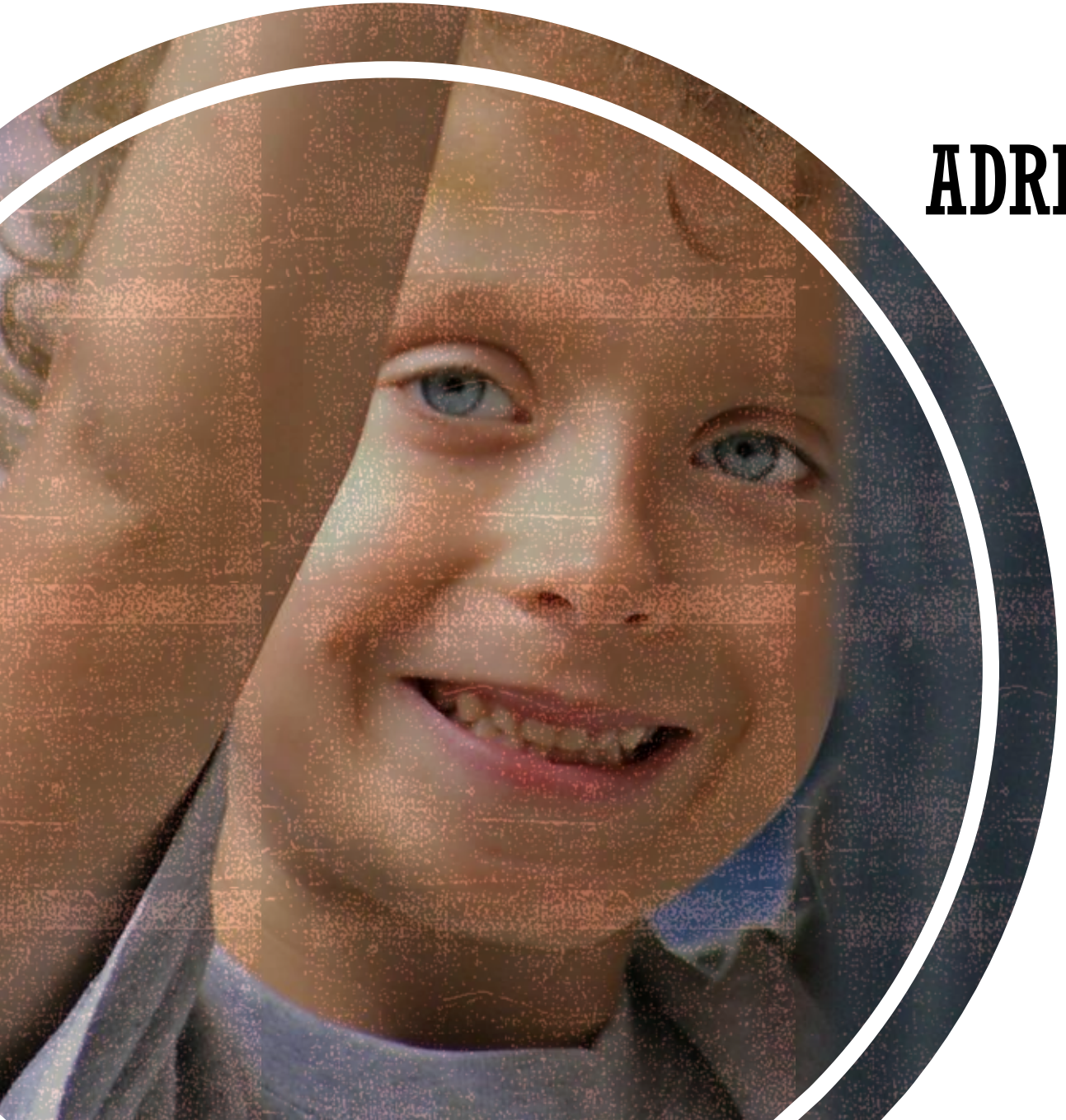
CONGENITAL ADRENAL HYPERPLASIA CASE

- CAH has relatively high FPR and FNR – molecular testing appealing
- *CYP21A2* gene in chromosomally complex region
  - 30kb deletion, gene conversion, pseudogene, etc.
- Phenotype is typically dictated by the less severe of the two variants

CONGENITAL ADRENAL HYPERPLASIA CASE

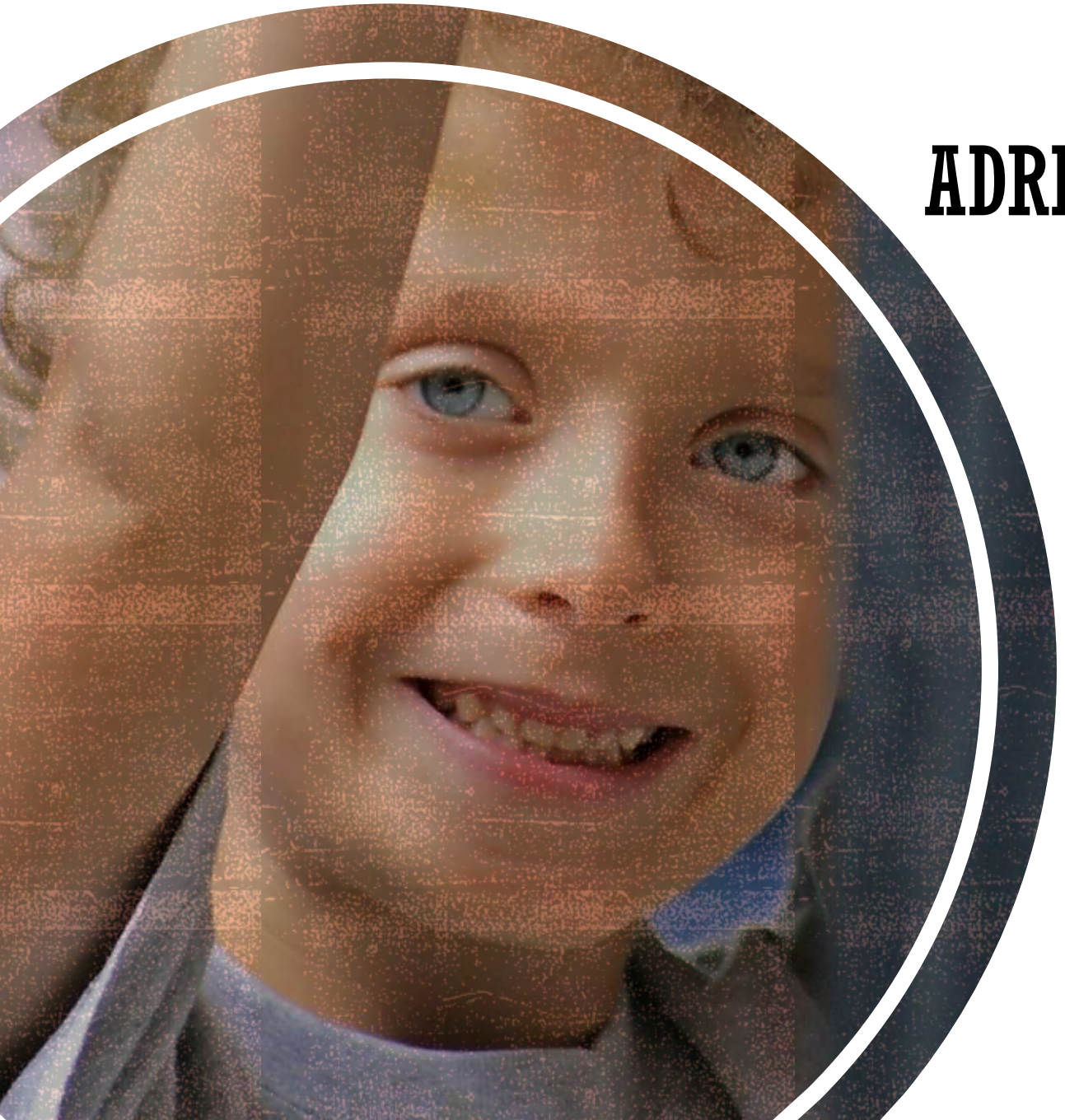


- 8 variants found
- Phasing done to determine variant configuration
- Patient actually has SW CAH due to NC variants being in *cis* with other variants



X-LINKED ADRENOLEUKODYSTROPHY CASE 1

- Infant screens positive on newborn screening with elevated C26:0-LPC
- A variant in *ABCD1* is detected by the screening program
- Child has two older brothers who were not screened for X-ALD



X-LINKED ADRENOLEUKODYSTROPHY CASE 1

- VLCFAs are drawn on mother and brothers
  - All have normal results
- *ABCD1* analysis is completed on the mother and she does **not have** the same variant identified in her son
- The variant identified in the child is a *de novo* variant. Other family members are not at risk.

X-LINKED ADRENOLEUKODYSTROPHY CASE 2

- Infant screens positive on newborn screening with very elevated C26:0-LPC
- No variant is found in *ABCD1*

X-LINKED ADRENOLEUKODYSTROPHY CASE 2

- VLCFAs are drawn on child and are elevated
- Peroxisomal panel is sent and returns with no variants found
- Mother, two maternal aunts, and two male cousins are also found to have elevated VLCFAs

X-LINKED ADRENOLEUKODYSTROPHY CASE 2

- Given apparent X-linked inheritance, the child is ultimately given a diagnosis of X-ALD even in the absence of an identifiable *ABCD1* variant and functional studies are sent

MPS I CASE

- Child is found to have near absent measurement of IDUA by the screening lab
- Other enzyme analyses (GAA, Biotinidase, GALT) were all normal
- 2nd tier GAG analysis is positive
- Child is seen clinically and is found to have a hernia
- Molecular testing only reveals one variant

MPS I CASE

- Specialist suspects MPS I and orders molecular testing clinically along with deletion/duplication testing
  - Del/Dup testing comes back normal (this is not surprising in MPS I!)
  - But… sequencing reveals **two** variants

MPS I CASE

- Program needs to go back to identify issue
  - Allele dropout is found to be cause of the one variant finding
  - Allele dropout = term for a failure of one allele to amplify. Patient is found to have a rare variant (polymorphism) at a primer site

THANK YOU AND QUESTIONS

agaviglio@cdc.gov