



# Exome / Genome Sequencing Result Interpretation & Clinical Correlation

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# Learning Objectives

Learners will be able to:



# Data generated by Exome / Genome

- Variants:

- CNV – copy number variation (typically > 1000bp)
- INDELs – insertion or deletion of a small number of base pairs ( 1 – 1000bp)
- SNV – single nucleotide variation

Coding or non-coding

- Coding variants :
  - Benign / Likely Benign (this includes population polymorphisms)
  - Pathogenic / Likely Pathogenic
  - Variants of Unknown Significance
- Non-coding variants
  - Impact a splice site
  - Impact a gene regulation site



# Data generated by Exome / Genome

- Variation that is more difficult to detect:
  - Genomic rearrangements
  - Nucleotide repeat disorders
  - Aneuploidy / chromosomal translocations
  - Mitochondrial genome is not always reported



# Copy Number Variation

- Mathematically inferred
  - For the information seekers: methodology applied
    - Read-Pair (RP) / Split-Read (SR) / Read-Depth (RD)/ Assembly (AS)
- Important limitations of ES
  - ~ 10% of exons not covered
  - Short read assembly creates limitations for variants used to assess CNVs
- Microarray has higher sensitivity - even more relevant for smaller CNV
- WES and SNP arrays can detect concordant gene-level alterations, especially those that are longer and well covered by multiple exons (for WES) or probes (for SNP)



# You have found a variant – now consider the evidence you have about the change


- Categories of Evidence

- Population Data (gnomad, Exac)
- Other Lab's Interpretations ("Reputable Source", Clinvar, HGMD)
- Computational/Predictive Data
- Functional Data (Pubmed, mechanism)
- Segregation Data
- De Novo?
- Trans/cis? (in trans?)
- The patient (do they have it?)



# ACMGG criteria for defining a variant

*Genetics in Medicine* volume 20, pages 918–926 (2018)

		BENIGN CRITERIA		PATHOGENIC CRITERIA			
Strength of evidence		Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Odds of Pathogenicity*		-18.7	-2.08	2.08	4.33	18.7	350.0
Evidence Category and Corresponding ACMG/AMP Codes	Population Data	BA1 <sup>+</sup> BS1 BS2			PM2	PS4	
	Allelic Evidence & Cosegregation Data	BS4	BP2 BP5	PP1 			
					PM3 PM6	PS2	
	Computation & Predictive Data		BP1 BP3 BP4 BP7	PP2 PP3	PM1 PM4 PM5	PS1	PVS1
	Functional Data	BS3				PS3	
	Other		BP6	PP4 PP5			



# Approaching a Variant

Previously reported in association with disease, or at all?

- May still be a VUS (even if it is “pathogenic”)
- How many times was it reported? What was the published or reported evidence? What were the phenotypes of the patients who had this variant?

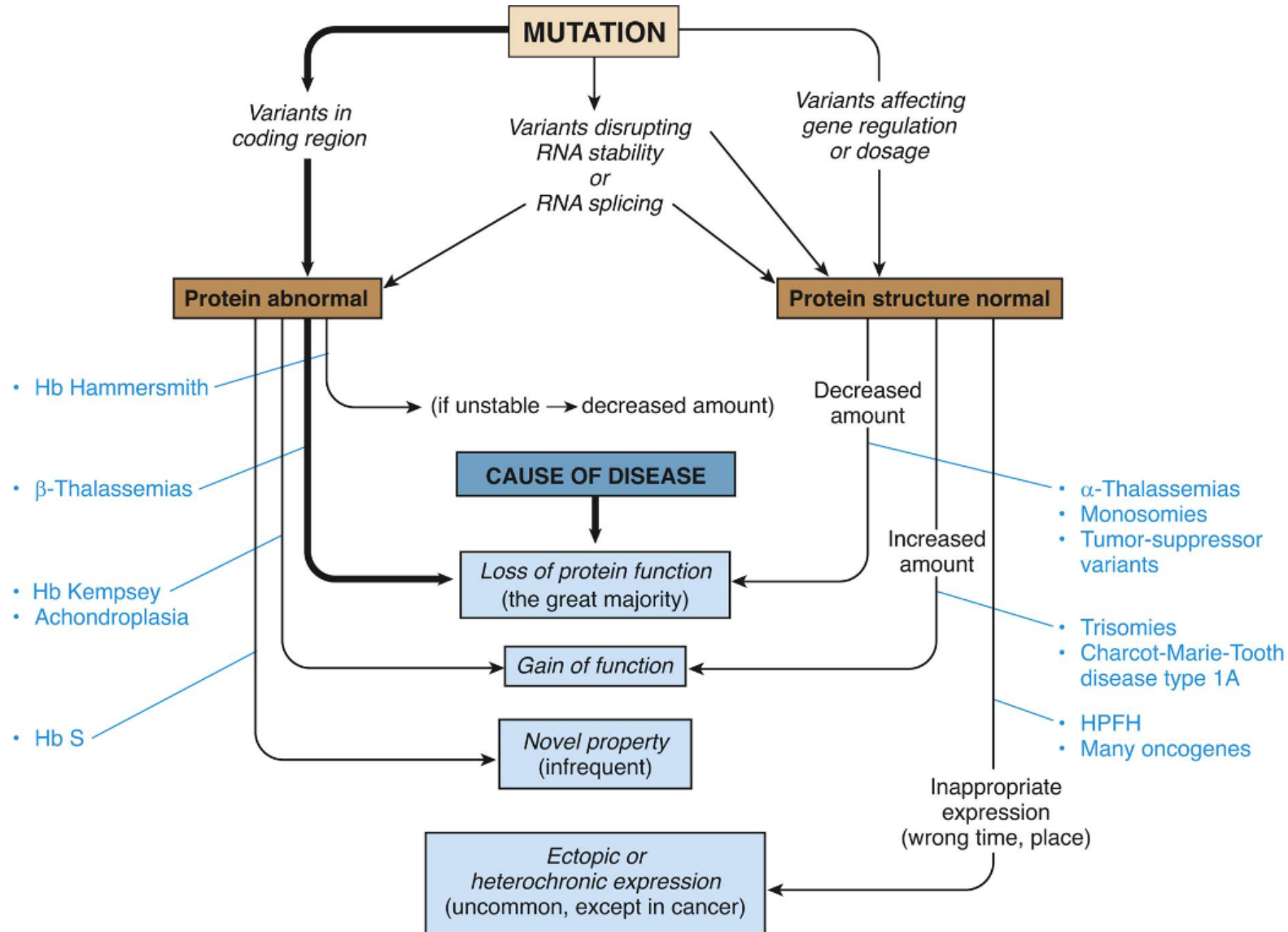
Variant frequency in population databases?

- If not in any databases, “private” = strong evidence
- If in database, is it too common?
  - Consider: Penetrance of disorder, age of onset, variable expressivity

If variant is too common: FULL STOP, likely benign



# The Molecular Basis of Genetic Disease





# Effect of Pathogenic Variants

- Loss of function
  - Gene dosage effect
  - Impact == degree of function lost – reduction vs complete loss
- Gain of function
  - Gene dosage
  - Enhanced activity / function of gene
- Novel properties
  - Altered property of protein – eg sickle cell
- Ectopic gene expression
  - Inappropriate gene expression :: timing vs tissue
  - More common in cancer / oncogenes
  - HbF – persistence of fetal Hb ~ abnormal regulatory elements



# Resources for additional investigation

- Call a friendly genetics professional – Lab, GC, Medical
- Explore for yourself:
  - gnomAD
  - ClinVar
  - Bioinformatic tools such as Varsome / Franklin
    - FGFR3 – G380R ( amino acid – can also use the base position change )
    - <https://varsome.com/variant/hg38/FGFR3%20G380R?annotation-mode=germline>
    - <https://franklin.genoox.com/clinical-db/variant/snp/chr4-1806119-G-A>



# OK it's a VUS... now what?

## Type of variant:

- Loss of function: more interesting
  - Premature stop, Frameshift, or canonical splice site (-/+ 1 or 2)
- Missense
- Non-frameshift del/dup or non-canonical splice site variant
- Intronic, UTR variant (untranslated region)

## Mechanism of disease

- Haploinsufficiency – lose 50% activity is a problem
- Complete LOF – need to lose almost all activity to have a problem
- Gain-of-function – increase in activity
- Dominant Negative – interferes with normal copy of gene

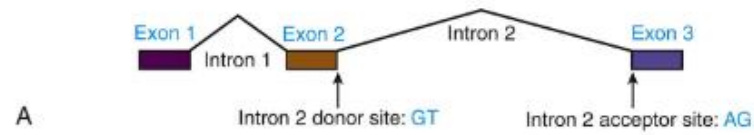


# Splice Variants

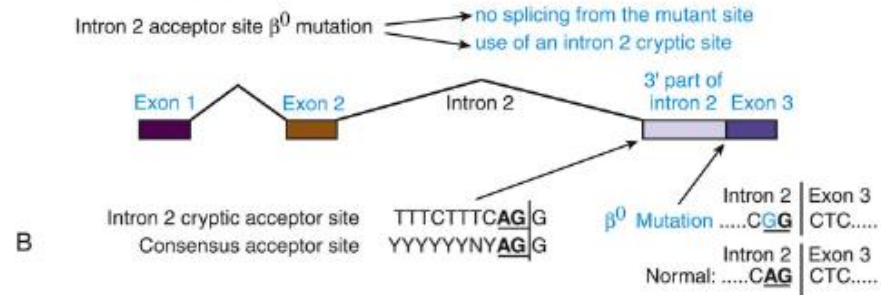
## Cryptic donor or acceptor splice sites

- Tools such as SpliceAI can help with predicting impact
- <https://spliceailookup.broadinstitute.org/>

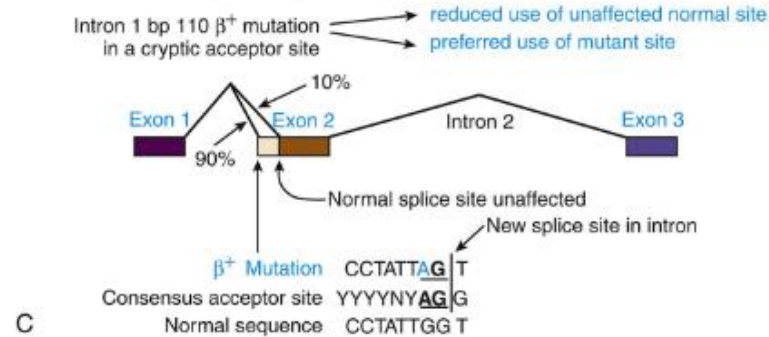
### Normal splicing pattern



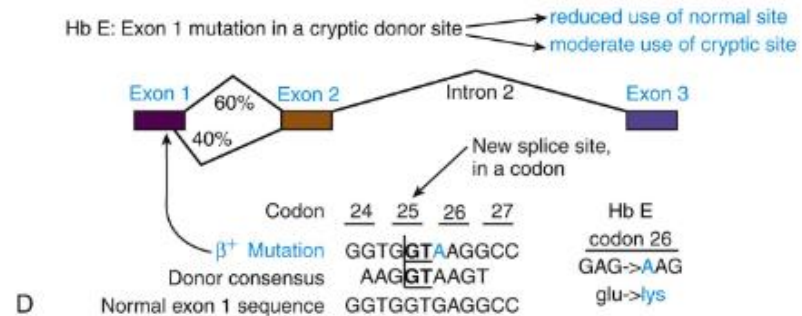
### Mutation destroying a normal splice acceptor site and activating a cryptic site



### Mutation creating a new splice acceptor site in an intron



### Mutation enhancing a cryptic splice donor site in an exon



# Report Errors and Provisional Reports

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Jim Weber, PhD

PreventionGenetics was acquired  
by Exact Sciences in 2021.

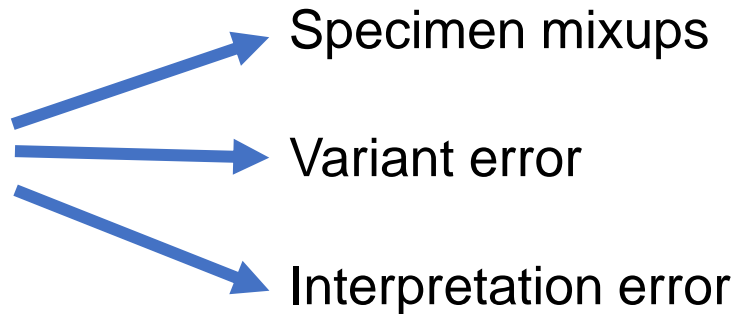
I am no longer employed by Exact,  
but I do still own some Exact stock.

# Report Errors and Provisional Reports

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Error (major) Rate  
for Final Reports

1/300 reports



Error Rate Depends On:

Variant type

Gene

Test

Lab

Geneticist

Provisional vs. Final Report

# Report Errors and Provisional Reports

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Lab	Reason(s) for Provisional Reports	Time (days) between Provisional and Final Reports
Lab A	Only final reports	NA
Lab B	<u>Written</u> preliminary reports when confirmation of a variant is required	8
Lab C	<u>Oral</u> provisional reports by GCs when confirmation of a variant is required Switching to written preliminary reports in about one month.	5

All genetic tests have limitations.

These results should be interpreted in context of clinical findings, family history and other laboratory data.