Harmonizing clinical interpretation of intragenic sequence and copy number variants in monogenic disease

INVITAE

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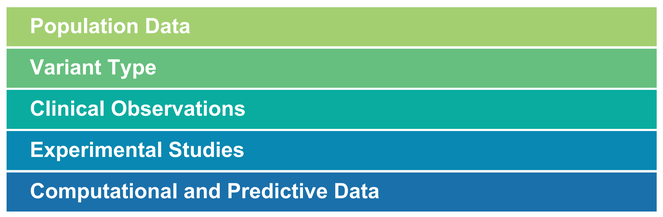
Introduction

- Intragenic copy number variant (CNV) detection is increasingly utilized in clinical genetic testing.
- The 2015 ACMG variant interpretation guidelines provided limited guidance for interpreting single/multi-exon deletions and do not address single/multi-exon duplications.
- Previously, we published the **Sherloc** variant interpretation schema based on the ACMG guidelines. Sherloc was iteratively refined based on the experiences of interpreting thousands of intragenic CNVs from a clinical cohort of over a hundred thousand individuals.
- These refinements have resulted in a unified variant interpretation system that allows for accurate, consistent, and efficient classifications for both sequence variants (SV) and intragenic CNVs.

Methods

- A key feature of Sherloc was the reorganization of weighted evidence-criteria into **categories** based on five **molecular and clinical genetics concepts**:



This allowed for the establishment of **complex relationships** within and between evidence categories.

Example relationships:

- Experimental data **trumps** *in silico* predictions.
- High allele frequency in the general population **modulates** the significance of a variant observations in a patient.
- Co-segregation of a variant with disease is **additive** for each additional family.

- Sherloc was expanded for interpretation of CNVs by:
  - Creation of additional evidence-criteria for **different types of CNVs**, and weighted *relative to* previously established criteria of similar concept.

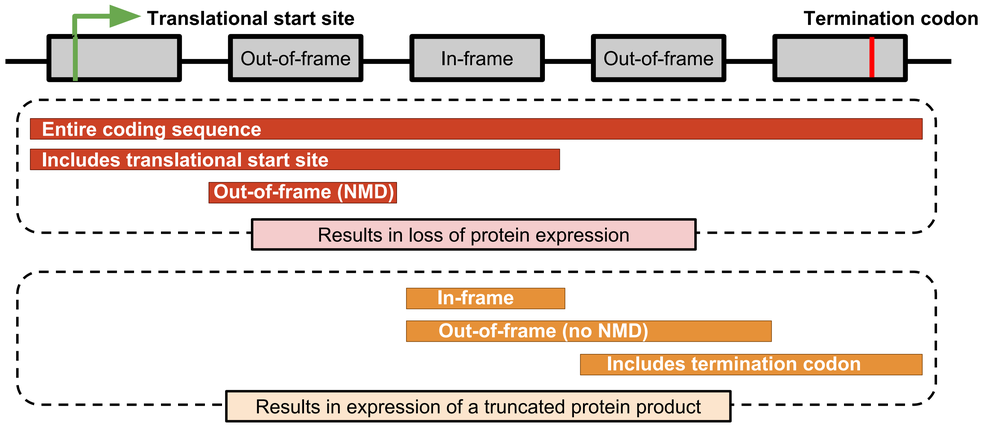
Examples:

A **whole-gene deletion is similar to a nonsense variant that leads to nonsense-mediated decay** since both abolish protein expression. These two criteria should be weighted the **same**.

A **duplication of an out-of-frame exon is similar to a frameshift that leads to nonsense-mediated decay** only if the duplication occurred in a tandem orientation. These two criteria should be weighted **similarly, but adjusted** for the possibility of the duplication event is not in tandem.

  - Inclusion of these CNV-related criteria within the **Variant Type** category to maintain established relationships with criteria in other categories.

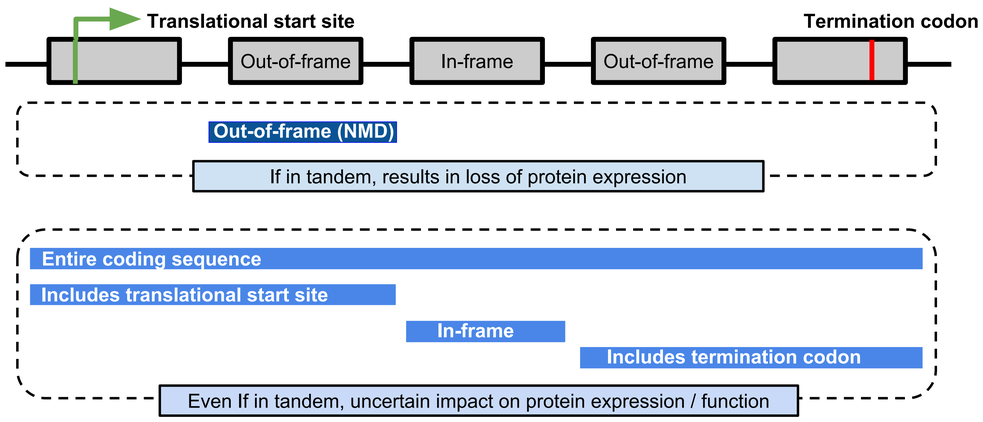
Types of Copy Number Loss Variants (Deletions)



Variant Type	Mechanism of disease is loss-of-function	Sherloc Score*
Whole gene deletion	**Yes**	**5 pts**
	No	2 pts
Include translational start site	**Yes**	**5 pts**
	No	2 pts
Out-of-frame exon(s), does not include penultimate coding exon	**Yes**	**5 pts**
	No	2 pts
Out-of-frame(s), includes penultimate coding exon	**Yes**	3 pts
	No	0 pts
In-frame exon(s)	**Yes**	3 pts
	No	0 pts
Includes termination codon	**Yes**	3 pts
	No	0 pts

* Sherloc score of 5 points corresponds to a classification of **Pathogenic** without requiring additional supporting evidence. A score of 4 points corresponds to a classification of **Likely Pathogenic.**

Types of Copy Number Gain Variants (Duplications)

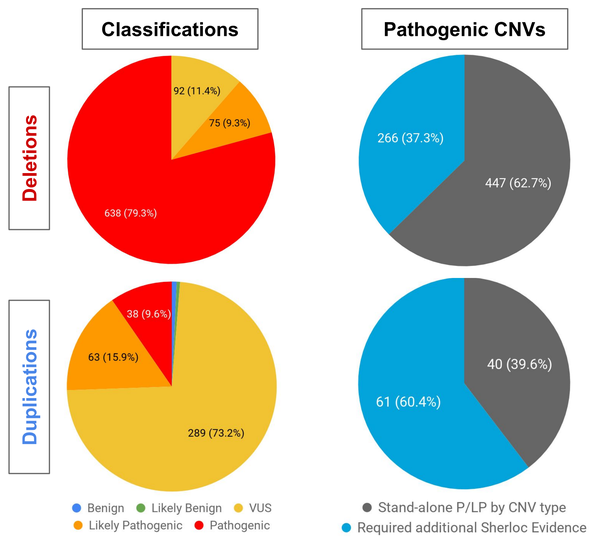


Variant Type	Mechanism of disease is loss-of-function	Sherloc Score*
Whole gene duplication	**Yes**	2 pts
	No	0 pts
Include translational start site	**Yes**	2 pts
	No	0 pts
Out-of-frame exon(s), does not include penultimate coding exon	**Yes**	**4 pts**
	No	0 pts
In-frame exon(s)	**Yes**	2 pts
	No	0 pts
Includes termination codon	**Yes**	2 pts
	No	0 pts

* Sherloc score of 5 points corresponds to a classification of **Pathogenic** without requiring additional supporting evidence. A score of 4 points corresponds to a classification of **Likely Pathogenic.**

The Impact of a Unified Interpretation System on a Clinical Cohort

- In a clinical cohort of 143,515 individuals, we observed 805 *unique* deletion CNVs and 395 *unique* duplication CNVs.
- 68% (814/1200) of them reached a classification of Pathogenic or Likely pathogenic (P/LP).
- Of these P/LP variants, 40% (327/814) would have been classified as a VUS had it not been for supporting evidence from other Sherloc categories that were originally created for SV interpretations, such as segregation and clinical observations.



Example: MLH1 Deletion (Exon 16-19)

Variant Type: **CNV Deletion that includes the termination codon** in a gene where molecular mechanism of disease has been established as **LOF**.

This is expected to result in a truncated MLH1 protein product.

Sherloc Score: 3 pts

Additional Sherloc evidence:

- **Segregation** in a family (1 pt)
- A single amino acid deletion within this region (p.Lys618del) has been classified as pathogenic, indicating that this CNV disrupts an **essential codon** (1.5 pts)

Final Classification:

Pathogenic (5.5 pts)

CONCLUSIONS

- The organizational structure of Sherloc, defined by molecular and clinical genetics concepts, allowed for seamless expansion of CNV-type evidence criteria, without perturbing any other component of this variant interpretation schema.
- The ability to apply previously-established Sherloc criteria from the other four categories allowed for a large fraction of variants to reach a classification of Pathogenic or Likely Pathogenic. This was particularly critical for duplication CNVs.

Disclosures: Authors are stockholders and employees of Invitae.