# The paperwork matters! Phenotypic information significantly impacts variant interpretation in hereditary cancer testing

Carolina Pardo, Daniela Iacoboni, Karl Erhard, Hio Chung Kang, Shu Huei Wang, Janita Thusberg, Rita Quintana, Jennifer Holle, Karen Ouyang, Scott T. Michalski, Shan Yang, Michael Anderson
Invitae, San Francisco, CA

## BACKGROUND

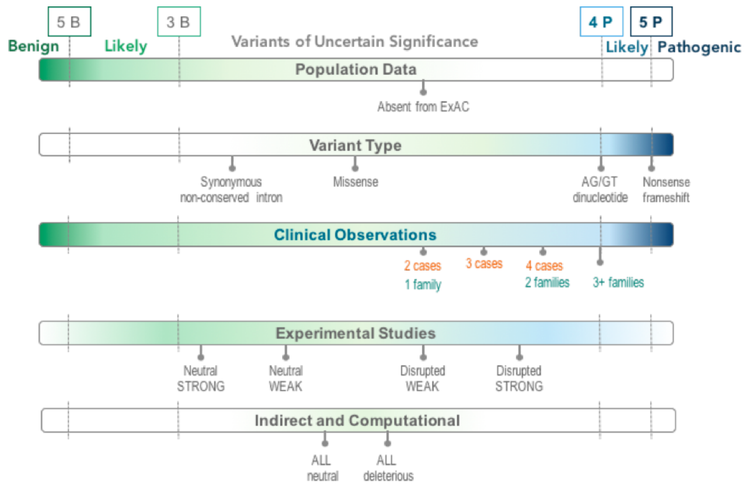
- The validity and utility of genetic testing require evidence-based, objective, and systematic variant interpretation.
- Well-described published clinical case reports and patients' phenotypic clinical information provided by the ordering clinician serve as the primary sources for case report data used in variant interpretation.
- As an extension of our laboratory's evidence-based variant classification framework, Sherloc, we developed point-based criteria for the objective inclusion of clinical information. As part of this process, we established a set of predefined clinical criteria for approximately 130 oncology genes.
- **In this study, we sought to determine the impact of incorporating critically-evaluated clinical phenotypic information into our variant classification system for over 100,000 patients undergoing testing for hereditary cancer.**

## METHODS

- A series of de-identified patients who underwent hereditary cancer testing over a 2-year period (September 2015 – November 2017) are included in this study.
- Only cases for which at least one of 32 pre-selected cancer genes were analyzed (*APC, BMPR1A, CASR, CDC73, CDH1, DICER1, EPCAM, FH, FLCN, MAX, MEN1, MLH1, MSH2, MSH6, NF1, NF2, PMS2, PTCH1, PTEN, RB1, RET, SDHB, SDHC, SDHD, SMAD4, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL*).
- These genes were selected because:
  - They are commonly requisitioned for hereditary cancer indications.
  - Internal clinical case report criteria have been developed, tested, and implemented in variant classification.
- Among this cohort of patients, Pathogenic and Likely Pathogenic (P/LP) variants for which clinical case report evidence was applied were selected.
- We assessed how frequently exclusion of this clinical evidence would have resulted in a classification of Variant of Uncertain Significance (VUS) instead of P/LP, possibly affecting clinical management of patients.

### Sherloc clinical criteria evidence

**Figure 1. Illustration of the Sherloc classification scoring thresholds and evidence categories.**




Among the five main evidence categories in Sherloc (Figure 1), the **Clinical Observations** sub-category contains evidence types related to case report criteria (i.e., compelling phenotypic presentations in a tested individual), segregation of the variant within a single family or multiple unrelated families, and de novo events (Table 1).

**Table 1. Sherloc clinical case reports evidence types.**

| Evidence category | Description of the evidence types | Pathogenic points |
|---|---|---|
| **Case reports** | 4 unrelated case reports | 3 |
| | 3 unrelated case reports | 2 |
| | 2 unrelated case reports | 1 |

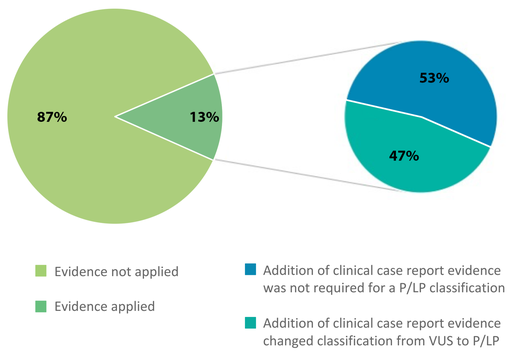
- The clinical case report evidence category has been further expanded into three sub-evidence types, allowing for the additive nature of evidence towards classifying a variant as Pathogenic (**5 pathogenic points**).
- For genes that have established consensus clinical diagnostic criteria, like *STK11*, *NF1*, *RB1*, *MEN1*, etc., our interpretation criteria are nearly identical to the consensus clinical diagnostic criteria.
- For genes that lack a formal consensus, such as *SDHB*, we have taken a rigorous, conservative approach in establishing internal criteria that considers age of onset of disease, phenotypic specificity, penetrance, prevalence, and the existence of phenocopies.

## RESULTS

### Impact of objective inclusion of clinical information on variant classification

**Figure 2. Inclusion of clinical case report evidence contributes to P/LP variant classification.**

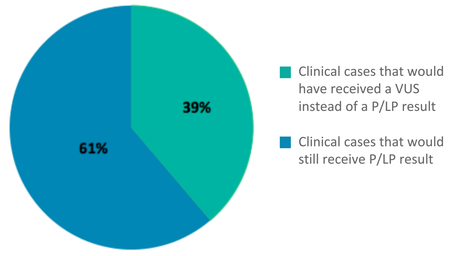
- P/LP variants were identified in 4,931/119,046 patients (4.1%) in our cohort.
- This represents a total of 2,062 unique P/LP variants.




- Clinical case report evidence was available and applied to 268 (13%) of the 2,062 unique variants.
- Inclusion of clinical case report evidence resulted in a P/LP classification in 126 of these variants (47%).

**Figure 3. Clinical cases where case report evidence was critical to obtain a P/LP test result.**

- Inclusion of clinical case report criteria evidence affected 929 (19%) cases in our cohort.
- 39% (360) of these patients received a positive P/LP result instead of a VUS result, due the inclusion of clinical case report criteria evidence during variant interpretation.




### Gene-specific impact of clinical information on variant classification

**Figure 4. Number of variants per gene for which clinical data inclusion resulted in P/LP classification.**

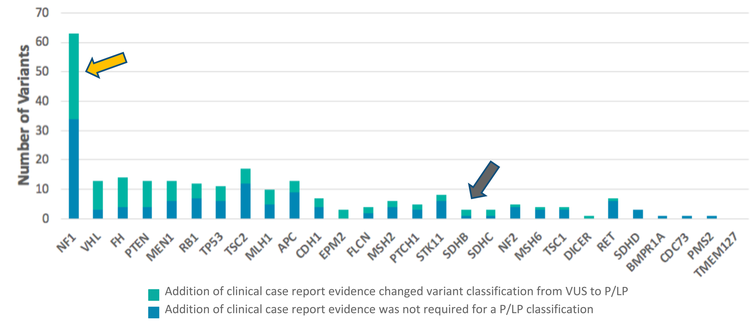
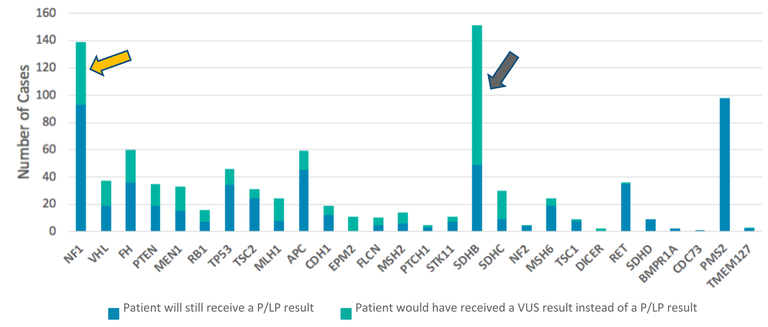



**Figure 5. Number of clinical cases per gene for which clinical data inclusion contributed to a P/LP test result.**




- To date, application of clinical data has contributed to P/LP variant classification in 72% of the analyzed genes in our cohort.
- Inclusion of clinical data in variant interpretation affected more variants in genes that have established consensus clinical diagnostic criteria, such as *NF1*, *VHL*, *FH*, *PTEN*, *MEN1*, *RB1*, *TP53*.
- Interestingly, inclusion of clinical data during variant interpretation affected more clinical cases with variants in genes that lack formal consensus clinical case criteria, such as *SDHB* and *SDHC*.

## CASE EXAMPLE

Clinician-provided patient information is particularly valuable as many classified variants are only observed in the clinical testing laboratory setting.

Here we illustrate a *FH* variant classified as LP due to the inclusion of clinician-provided, detailed phenotypic information.

**Invitae *FH* case report criteria**

An individual MUST fulfill the Major feature, OR 2 or more of the Minor features:

**MAJOR feature:**
2 or more cutaneous leiomyomas - with at least 1 histologically confirmed.

**MINOR features:**
a. 2 or more uterine leiomyomas (or fibroids) before age 40 years.
b. Renal cancer (histopathological type papillary, tubulo papillary, or collecting-duct carcinoma).
c. A variant-positive or un-genotyped first degree relative who fulfills the major feature OR minor feature b.

**Table 2. Pathogenic evidence for LP variant in *FH***

| Evidence category | Description of evidence type | Pathogenic points |
|---|---|---|
| **Population Data** | Absent in general population | 1 |
| **Experimental Studies** | Protein function disrupted: weak functional evidence | 1 |
| **Clinical Observations** | Weak segregation with disease: 3 affected carriers with skin leiomyomas, uterine leiomyomas and/or renal cell carcinomas, and 4 unaffected non-carriers. | 1 |
| **Clinical Observations** | 2 unrelated case reports (Invitae): 1. Proband with 3 pathology confirmed leiomyomas. Mother and maternal grandmother reported to have leiomyomas. 2. Proband with multiple cutaneous leiomyomas on limbs and back (pathology confirmed) in her 20's, and 1 uterine fibroid before 40 years old. | 1 |
| | **Classification: Likely Pathogenic** | **4** |

## CONCLUSIONS

- Patient phenotypic data can play a critical role in the variant interpretation process.
- We demonstrate that a substantial proportion of our P/LP cases would have otherwise remained VUSs, suggesting our current pool of VUSs hold the potential for reclassification considering additional case report evidence.
- Ordering providers may profoundly influence variant classification by sharing complete, accurate personal and family history data.

**Disclosures:** All authors are stockholders and employees of Invitae.