Paperwork matters! The importance of clinical phenotype information in variant interpretation

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Abstract

The validity and utility of hereditary germline testing require that variant classifications be evidence-based, objective, and systematic. As genetic testing becomes available to a larger percentage of the population, detailed clinical information about patients and their family members becomes increasingly relevant for variant classification. Although sufficient clinical information is often provided in well-described case reports in the published literature, most classified variants are observed only in the clinical testing laboratory setting. Therefore, the ordering clinician becomes the sole source of phenotypic data, as provided on the test requisition form. To objectively incorporate this clinical data into our laboratory's evidence-based variant classification framework called Sherloc, we defined point-based criteria and usage rules allowing us to evaluate the following:

- a patient's clinical phenotype
- variant segregation in families
- variant *de novo* status

As part of this process, we developed a set of predefined clinical criteria for ~130 oncology genes. For genes such as *NF2* and *STK11*, our interpretation criteria are nearly identical to the consensus clinical diagnostic criteria. For genes that lack a formal consensus, such as *SDHB*, we took a rigorous, conservative approach in establishing internal criteria that considers age of onset, phenotypic specificity, penetrance, prevalence, and the existence of phenocopies. The application of our method is illustrated in cases of *STK11* and *SDHB* variants, in which detailed phenotypic information provided by the clinician impacts variant classification. These results highlight the need for ordering providers to share detailed clinical patient information, as it may influence variant classification and ultimately, clinical care.

Sherloc clinical criteria evidence

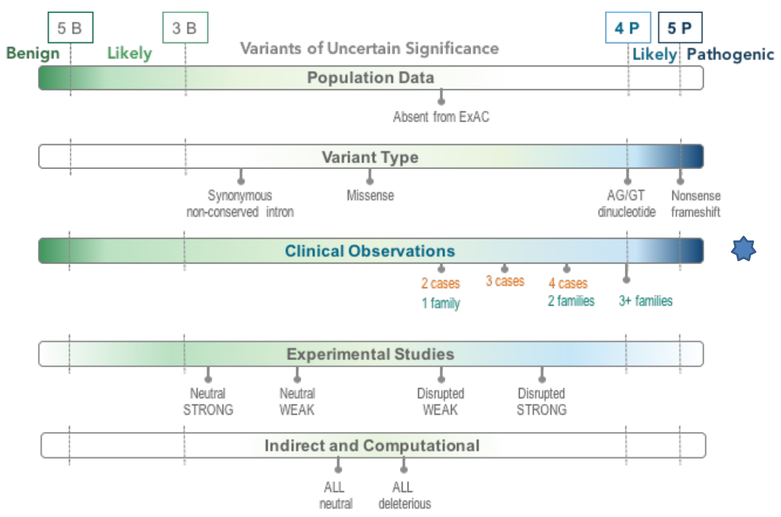


Figure 1. Illustration of the Sherloc classification scoring thresholds and evidence categories.

Among the five main evidence categories in Sherloc (Figure 1), the **Clinical Observations** category ( ) contains evidence types related to case report criteria (i.e., compelling phenotypic presentations in a tested individual), co-segregation of the variant within a single family or multiple unrelated families, and *de novo* events (Table 1). Each evidence type has been further expanded into two to three sub-evidence types, allowing for the additive nature of evidence towards classifying a variant as pathogenic (**5 pathogenic points**).[1]

Table 1. Sherloc case reports, segregation, and *de novo* evidence types.

Evidence type	Description of the sub-evidence types	Pathogenic points
Case reports	4 unrelated case reports	3
	3 unrelated case reports	2
	2 unrelated case reports	1
Segregation	Strong segregation with disease (≥ 10 informative individuals from 2 or more families)	4
	Moderate segregation with disease (≥ 6 informative individuals from 2 or more families)	2.5
	Weak segregation with disease (≥ 3 informative individuals from 1 or more families)	1
De novo	*De novo* with confirmed paternity/maternity	4
	De novo without confirmed paternity/maternity	2

Our case report criteria often mimic consensus diagnostic/testing criteria

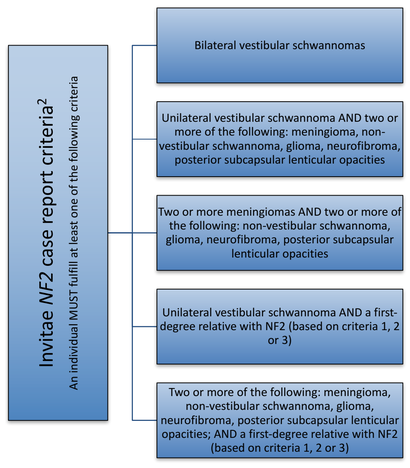


Figure 2. Invitae *NF2* case report criteria.

Like *NF2*, our case report criteria are very similar to the consensus diagnostic or testing criteria for:

BLM, *BMPR1A*, *CDC73*, *CDH1*, *DICER1*, *DIS3L2*, *FH*, *FLCN*, *KIT*, *MEN1*, *NF1*, *PTCH1*, *PTEN*, *RB1*, *RET*, *SMAD4*, *STK11*, *TP53*, *TSC1*, *TSC2*, *VHL*, and genes associated with Diamond-Blackfan anemia, Fanconi anemia, and Lynch syndrome.

Detailed clinical information leads to more accurate variant classifications

We received a sample from a 29-year-old individual with mucocutaneous macules and history of hamartomatous polyps. Her two children also had mucocutaneous macules and multiple paternal aunts and uncles reportedly had cancers associated with Peutz-Jeghers syndrome (PJS). Multigene panel testing revealed the *STK11* variant, c.526G>A (p.Asp176Asn).

Applying our case report criteria to this patient increased the total number of case reports to 3 (including two families identified in peer-reviewed publications).[4-7] For this *STK11* variant, its pathogenic classification results principally from the detailed clinical phenotypic information available, and the use of case reports and segregation evidence (Figures 3 and 4, Table 2).

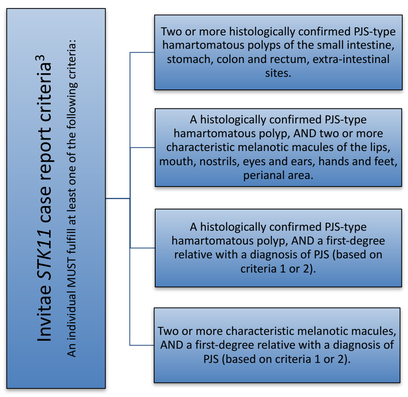


Figure 3. Invitae *STK11* case report criteria.

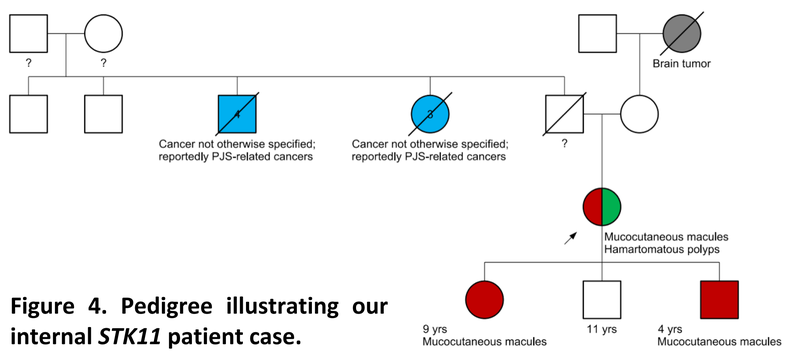


Figure 4. Pedigree illustrating our internal *STK11* patient case.

Table 2. Pathogenic evidence for the *STK11* variant, c.526G>A (p.Asp176Asn).

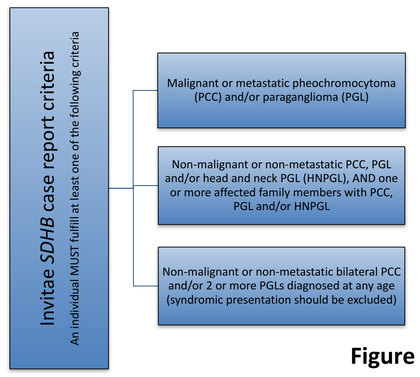
Evidence category	Description of the evidence types	Points
Population data	Absent in ExAC, but 10-80% of individuals have 20X coverage	0.5
Computational predictions	Protein predictions: conflicting or insufficient data	0
Molecular studies	Protein function disrupted: strong functional evidence	2.5
Case reports	**Three unrelated case reports** 1. Familial case of PJS with the proband having melanotic macules, PJS-polyps, and colon cancer.[4-6] Familial case of PJS with the proband and mother having melanotic macules and PJS-polyps.[7] 2. Familial case where the proband has multiple undescribed hamartomatous polyps, melanotic macules, and a family history of PJS (Invitae).	**2**
Segregation	**Weak segregation with disease.** Five affected carriers with melanotic macules and/or PJS-type hamartomatous polyps, and eight unaffected non-carriers.[4-6]	**1**
	Classification: Pathogenic	**6.0**

Developing case report criteria in the absence of consensus diagnostic criteria

For *SDHB*, which lacks formal consensus criteria, we established internal criteria based on prevalence, penetrance, age of onset, specificity of the phenotype, and known phenocopies for causing pheochromocytoma (PCC) and/or paraganglioma (PGL) (Table 3, Figure 5).

Table 3. Clinical data considered in developing the *SDHB* case report criteria.[8-12]

Clinical data considerations	Hereditary PGL / PCC	Sporadic PGL / PCC
Prevalence	~25% of all PGL / PCC cases	3-8 in 1 million cases per year
Peak incidence	24.9 years	43.9 years
***SDHB*-related features**		
Penetrance, by age 50 years	50-77% (overall ~50%)	
Mean age at diagnosis	~28 years (26-34 years)	~47 years (44-50 years)
Malignancy risk	34-97%	10-17%
SDHB mutation detection rate	17-100%	5.8% (sporadic or unselected)



Phenocopies such as *SDHA*, *SDHAF2*, *SDHC*, *SDHD*, *MAX*, *TMEM127*, *MAX*, *RET*, *NF1,* and *VHL* should be tested.

Figure 5. Invitae *SDHB* case report criteria.

In the case of the *SDHB* variant, c.293G>A (p.Cys98Tyr), the clinical data is highly suggestive of the variant being disease causing. However, further information is needed before making that assertion (Table 4).

Table 4. Pathogenic evidence for the *SDHB* variant, c.293G>A (p.Cys98Tyr).

Evidence category	Description of the evidence types	Points
Population data	Absent from the general population	1
Computational predictions	General protein predictions – all deleterious	0.5
Segregation	**Weak segregation with disease** Segregated with one to two extra-adrenal PGLs in four family members[13]	**1**
Case reports	**1 case report** Proband with a jugular PGL at 39 years old, and three family members with single or multiple PGLs[13-14]	**0**
Insufficient case reports	**Observed in patients but insufficient evidence** 1. Proband with an abdominal PGL at age 14 years[15] 2. Proband with a PGL in their 20s (Invitae)	**0**
	Classification: Pathogenic	**2.5**

Conclusions

- The systematic inclusion of clinical criteria allows us to objectively use patients' phenotypic information in variant classification and reclassification.
- Providing complete family history information and phenotypic details may make the difference in the classification of a variant. Clinicians are encouraged to include clear, concise clinical information in their test orders.
- Variant classification is an ongoing process. Variants that are currently classified as uncertain significance may be reclassified in the future based on phenotypic information provided by ordering clinicians.
- Key opinion leaders recognize the importance of a systematic approach such as ours, and suggest that future versions of the ACMG ISV Guidelines may consider a semi-quantitative approach such as the one used in Sherloc.[16]

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