Show me the phenotype: The ordering clinician's role in genetic variant interpretation for primary immunodeficiency diseases

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BACKGROUND

The rapid pace of new gene discovery and phenotype expansion for Primary Immunodeficiency Diseases (PIDDs) creates challenges for genetic testing and variant interpretation. Whereas well-described clinical case reports in published literature have traditionally served as the source of phenotypic data used for variant interpretation, for PIDDs the causal variants are often private to the patient's family and thus the sole source of phenotypic information for a novel genetic variant is frequently the history provided by the clinician on the test requisition form. Taking into account such heterogeneous information during variant interpretation requires establishing objective criteria for its inclusion as part of the variant interpretation process.

CLINICAL DATA IN VARIANT INTERPRETATION

- We adapted our laboratory's pre-existing, evidence-based variant classification framework, called Sherloc[1] (Figure 1) by developing point-based criteria for the inclusion of clinical information such as a patient's phenotype, familial segregation patterns, and whether the variant is inherited or de novo in the patient.

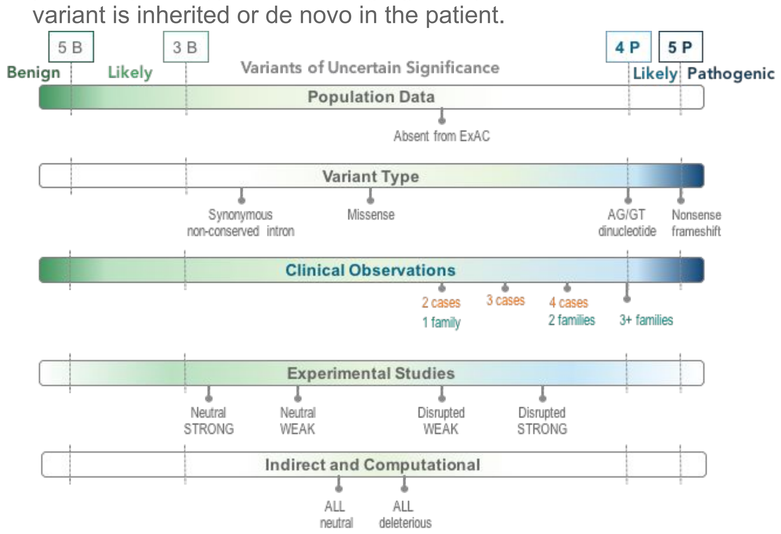

Figure 1. Illustration of the Sherloc classification scoring thresholds and evidence categories.

- The Clinical Observations subcategory of the Sherloc framework is further expanded into the following evidence types (Table 1):

Evidence Category	Description of Clinical Evidence Types	Pathogenic Points
Segregation: likelihood of segregation with disease by random chance	Weak segregation with disease (prob. of co-segregation is ≤0.25)	1
	Moderate segregation with disease (prob. of co-segregation is ≤0.0625)	2.5
	Strong segregation with disease (prob. of co-segregation is ≤0.03125)	4
Case Reports: clinical sensitivity ≥ 25%	Observed in patients but does not meet clinical criteria	0
	1 case report meeting clinical criteria	0
	2 unrelated case reports meeting clinical criteria	1
	3 unrelated case reports meeting clinical criteria	2
	4 unrelated case reports meeting clinical criteria	3
Pathognomonics: clinical sensitivity ≥ 75%	Hemi- or homozygous variant in pathognomonic gene	2
	Heterozygous in pathognomonic gene (Dominant only)	1
	Rare htz variant co-occurring with LP/P in pathognomonic gene	1.5
De novo	De novo with confirmed paternity/maternity	3
	De novo without confirmed paternity/maternity	2
Co-occurrence	Variant in trans with LP/P variant in affected individual	1

Table 1. Clinical criteria evidence types utilized in the Sherloc classification system

- To systematically include such phenotypic data for PIDD patients, we defined clinical criteria for 154 PIDD genes.
- We analyzed the clinical information provided from ordering clinicians, and the criteria utilized in the variant interpretation of immunological genetic tests ordered from April 2017 to October 2018.

CASE CRITERIA FOR PIDDs:

- Our case report criteria are derived from expert or consensus guidelines for the clinical diagnoses of PIDDs with modifications for use in the clinical lab setting (Table 2):

Condition	Clinical Criteria	Source
Autoimmune lymphoproliferative syndrome	1. Chronic, nonmalignant, noninfectious lymphadenopathy or splenomegaly or both 2. Elevated DNT cells (≥1.5% of total lymphocytes or 2.5% of CD3+ lymphocytes) in the setting of normal or elevated lymphocyte counts. 3. Defective lymphocyte apoptosis (in 2 separate assays)	Adapted from PMID: 20538792
Chronic Granulomatous Disease	1. Absent/significantly decreased respiratory burst (NBT or DHR, measured at least twice) AND 2. At least one of the following: -deep seated infection due to bacteria and/or fungi (abscesses, osteomyelitis, lymphadenitis) -recurrent pneumonia -lymphadenopathy and/or hepatomegaly and/or splenomegaly -obstructing/diffuse granulomata (gastrointestinal or urogenital tract) -chronic inflammatory manifestations (colitis, liver abscess and fistula formation) -failure to thrive -affected family member	PMID: 30776527
Severe Combined Immunodeficiency (typical)	Case Report: a patient less than two years of age with either (a) an absolute CD3 T cell count of less than 300/mm3, or (b) an absolute CD3 T cell count of greater than 300/mm3 with absent naïve CD3/CD45RA T cells.	Adapted from PMID: 20301656, 26255240, 23776382
Adenosine deaminase deficiency	ADA Pathognomonic: <1% normal erythrocyte ADA activity in un-transfused patients	
X-linked SCID	IL2RG Pathognomonic: T- B+NK- immunophenotype by flow cytometry AND JAK3 must have been tested and without pathogenic mutations	

Table 2. Examples of case report and pathognomonic criteria utilized at Invitae.

CLINICAL DATA RESULTS IN MORE ACCURATE VARIANT CLASSIFICATIONS

- Of the 4,057 immunology genetic tests ordered during the studied period, information about the patient's clinical history was provided in 70% of orders and family history information was provided in 17% of orders.
- Ten variants of uncertain significance were reclassified following receipt of further clinical information or testing of additional relatives.
- In addition, 35 "suspicious" variants of uncertain significance were identified in which one or two additional patient case reports would allow for reclassification from uncertain significance to P/LP.
- There were 3,868 variants identified in the 154 genes for we which developed case report criteria. Of those, 370 (10%) were classified as pathogenic or likely pathogenic (P/LP).

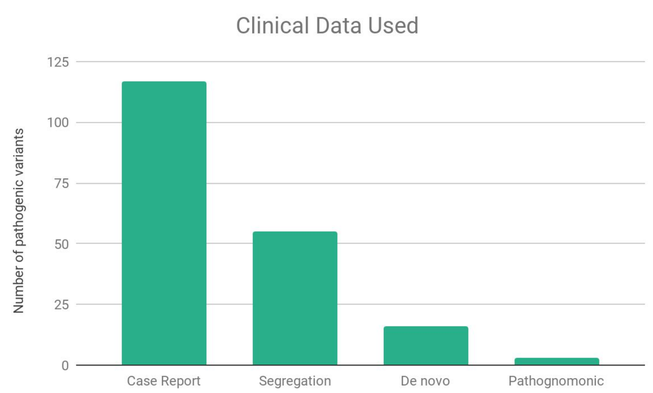

Figure 2. Clinical criteria used in the classification of P/LP variants.

- Information from case report descriptions, segregation patterns, and de novo status were applied for 32%,15% and 4% of P/LP variants, respectively. Pathognomonic criteria were utilized in 3 cases (Figure 2).
- Ten variants of uncertain significance were reclassified following receipt of further clinical information or testing of additional relatives.
- In addition, 35 "suspicious" variants of uncertain significance were identified in which one or two additional patient case reports would allow for reclassification from uncertain significance to P/LP.

CASE EXAMPLES

- Case 1. We received samples from five siblings affected with symptoms of CGD: achromobacter cellulitis, MSSA septicemia, Burkholderia gladioli infection, adenitis, and abnormal DHR assays (Figure 3). Using the provided clinical information and the CGD criteria outlined in Table 2, we were able to classify the c.1702G>A (p.Glu568Lys) variant in CYBB, which was identified in all siblings tested, as Pathogenic (Table 3).

CYBB Variant	Classification without clinical information from this family	Classification with clinical information from this family
c.1702G>A (p.Glu568Lys)	• Rare in the population (0.5) • Strong functional evidence (2.5) • 1 case report in literature (0) 3 pt VUS	• Rare in the population (0.5) • Strong functional evidence (2.5) • 2 unrelated case reports, literature & family (1) • Weak segregation with disease (1) 5 pt Pathogenic

Table 3. Evidence used in classification of E568K variant in CYBB

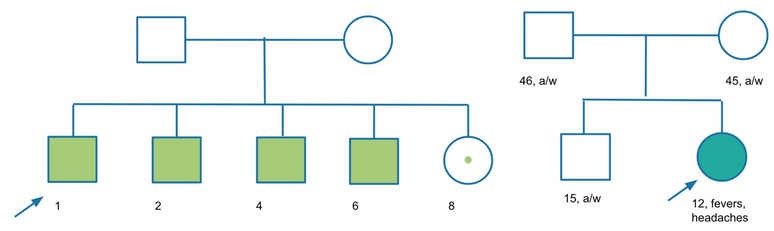

Figure 3. Pedigree from proband in case 1.

Figure 4. Pedigree from proband in case 2.

- Case 2. We received a sample from a 12-year-old female patient with fevers and headaches (Figure 3).
- Two variants in MVK with unknown phase were identified. The clinical information provided was suspicious for MVK but not specific enough to meet case report criteria. Both variants were initially classified as VUS. Following receipt of further clinical information, including mevalonic acid levels and parental samples, we were able to determine the variants were on opposite chromosomes and reclassify both VUSes as Likely Pathogenic.

MVK Variant	Classification before clinical data	Classification after clinical data
c.1139A>G (p.His380Arg)	• Rare in the population (0.5) • 4 unrelated case reports (3) 3.5 pt VUS	• Rare in the population (0.5) • 4 unrelated case reports (3) • In trans with LP variant (this case) 4.5 pt Likely Pathogenic
c.830G>A (p.Arg277His)	• Rare in the population (0.5) • In trans with P variant in literature (1) • Weak segregation with disease (1) 2.5 pt VUS	• Rare in the population (0.5) • In trans with P variant in literature (1) • Weak segregation with disease (1) • Patient with positive enzymatic or protein assay results (1.5) 4 pt Likely Pathogenic

Table 4. Evidence used in classification of H380R and R277H variants in MVK

CONCLUSIONS

- **The clinical phenotype and family history data of patients with PIDDs is valuable and necessary for accurate variant interpretation**
- **Providing good quality clinical information to the genetic testing laboratory at the time of sample submission is the most efficient way to insure the appropriate interpretation of genetic variants**
- **Follow up family studies, laboratory results, and new clinical information can result in the reclassification of variants of uncertain significance to likely pathogenic.**

References: 1. Nykamp K, Anderson M, Powers M *et al*. Sherloc: a comprehensive refinement of the ACMG variant classification criteria. *Genet Med*. 2017;19(10):1105-17.

Disclosures: All of the authors are stockholders in and employees of Invitae.