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Atypical risk variants in oncology and rare disease: Findings from a review of laboratory case studies and > 6000 ClinVar submissions

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BACKGROUND

INTRODUCTION
Interpretation of atypical risk variants (ARVs) such as pathogenic low penetrance (PLP) and hypomorphic variants is challenging for diagnostic laboratories, clinicians, genetic counselors, and patients. Our field lacks guidance on how to identify, classify and describe them. These terms can influence weight applied to a particular line of evidence, or whether variants are reported at all. These variants also contribute to conflicting classifications in ClinVar which may impact medical actionability and patient care. There is no reliable estimate of how often ARVs are encountered by diagnostic labs in practice.

PURPOSE
We reviewed laboratory cases from oncology and rare disease, along with ClinVar submissions, to identify hallmarks of ARVs and call for improved guidance on terminology, variant classification and reporting.

METHODS

ONCOLOGY AND RARE DISEASE CASE STUDIES
We reviewed select candidate ARVs identified in our diagnostic laboratory including variants in cancer-associated genes, such as *BRCA2, MEN1, MLH1,* and *TSC2*. Variants were also assessed in the rare disease genes *MYBPC3, SHQ1, PIGG,* and *KIAA0586*.
We compared evidence prompting consideration for atypical risk and evaluated evidence applied in the final classification of each variant under the current 2015 ACMG guidelines. All final classifications and reporting terms were approved by laboratory directors.

CLINVAR SEARCH FOR ARVs
We also analyzed search results for "hypomorphic" , "low penetrance" and "atypical risk" in ClinVar to identify commonalities and discrepancies in terminology, interpretation, and reporting of ARVs.

RESULTS

VARIANT	*BRCA2* c.7878G>C p.W2626C	*MEN1* c.652C>T p.R218W	*MLH1* c.1937A>G p.Y646C	*TSC2* c.3598C>T p.R1200W
MAF (gnomAD v2 max subpop)	0.0017%	Absent	0.006974%	0. 006486%
PHENOTYPE(S)	AD HBOC AR FA	AD Multiple endocrine neoplasia and/or FIHP	AD Lynch syndrome AR CMMRD	AD Tuberous sclerosis complex
ARV LANGUAGE:	Y "…may be hypomorphic and thus, carriers of this variant and their families may present with reduced risks, and not with the typical clinical characteristics of a high-risk pathogenic *BRCA2* alteration. As risk estimates are unknown at this time, clinical correlation is advised. "	Y "…carriers of this variant and their families may present reduced risks, clinical correlation is advised." "…RNA studies have demonstrated that this alteration results in an incomplete splice defect"	N "This alteration has been reported in multiple individuals with clinical histories suspicious for Lynch syndrome, although tumor test results have been inconsistent …Functional analyses of the p.Y646C variant have produced conflicting results."	Y "…carriers and families have been shown to present with a milder phenotype than classic *TSC1/2* pathogenic mutations…a pathogenic mutation associated with reduced penetrance compared to other *TSC2* mutations."
ACMG EVIDENCE CODES	**AD**: PS4_PC, PP3, PS3 **AR**: PM3, PP3, PS3	**AD**: PS4_PC, PS3_RNA	**AD/AR**: PP3, PM1	**AD**: PS4_PC, PP1, PS3
FINAL AMBRY CLASSIFICATION	Pathogenic	Pathogenic	VUS	Pathogenic
CLINVAR CLASSIFICATION	P or LP (VariationID: 38125)	Conflicting P, LP, VUS (VariationID: 200976)	Conflicting P, VUS, LB (VariationID: 36545)	Pathogenic (VariationID: 49770)

Table 1 – Oncology panel variants assessed at Ambry where ARV language was considered. Atypical language was omitted from VUSs due to insufficient evidence. As all variants exceeded PM2, terminal classifications were based on published experimental data, internal RNA data, or enrichment among internal cases compared to population databases.

VARIANT	*MYBPC3* c.1828G>C p.D610H	*KIAA0586* c.392delG p.R131Kfs*4	*PIGG* c.1515G>A p.W505*	*SHQ1* c.997C>G p.L333V
MAF (gAD v2 max subpop)	0.0086%	0.8335%	0.1215% (2 homozygotes in v4)	0.02506%
PHENOTYPE	AD/AR HCM	AR Joubert Syndrome	AR glycosylphosphatidyl-inositol deficiency	AR Neurodevelopmental disorder
ARV LANGUAGE USED?	N	Y "demonstrated reduced penetrance…"	Y "The presence of homozygotes in population databases and the variable expressivity may indicate that this variant is associated with reduced penetrance and/or severity…"	N
EVIDENCE CODES	**AD/AR:** PS4_PC, PP3	**AR:** BS1, PVS1, PP1, PM3	**AR**: BS1, BS2, PM3, PVS1	**AR**: PM3, PP1, PP3, PS3
AMBRY CLASSIFICATION	VUS	Pathogenic	Pathogenic	Pathogenic
CLINVAR	Conflicting P, LP, VUS (VariationID: 42576)	P or LP (VariationID: 204593)	P or LP (VariationID: 476318)	LP (VariationID: 2687777)

Table 2 – Rare disease WES variants assessed at Ambry where ARV language was considered. Atypical language was omitted from VUSs due to insufficient evidence. As all variants exceeded PM2, terminal classifications relied on published experimental evidence and/or relative enrichment among internal cases compared to population databases.

VARIANT	*MYH7* c.4377G>T (p.Lys1459Asn)	*CPT1A* c.1436C>T (p.Pro479Leu)	*BRCA2* c.9699_9702del (p.Cys3233fs)
MAF (gAD v2 max subpop)	0.05%	0.0830%	0.0397%
PHENOTYPE	AD HCM	AR CPT1A Deficiency	AD HBOC AR FA
ARV LANGUAGE USED?	Ambry: N Others: "Atypical presentation…this gene has incomplete penetrance."	Ambry: N Others: "Atypical presentation…Hypomorphic allele with reduced penetrance"	Ambry: Y "May be hypomorphic and carriers of this variant and their families may present with reduced risks." Others: "May show reduced penetrance compared to a traditional pathogenic BRCA2 variant…atypical Fanconi anemia"
AMBRY EVIDENCE CODES	BS1, BP4	PM3, PP3	PVS1, PS4_PC
AMBRY CLASSIFICATION	LB	Pathogenic	Pathogenic
CLINVAR	Conflicting; LB by ClinGen EP (VariationID: 43012)	Pathogenic (VariationID: 65644)	Conflicting; LP by ClinGen EP (VariationID: 38260)

Table 3 – Example variants in cardiology, rare disease and oncology interchangeably described using Hypomorphic, Pathogenic Low Penetrance and/or Atypical terminology in ClinVar.

Are any terms consistently used for specific variant classifications in ClinVar?

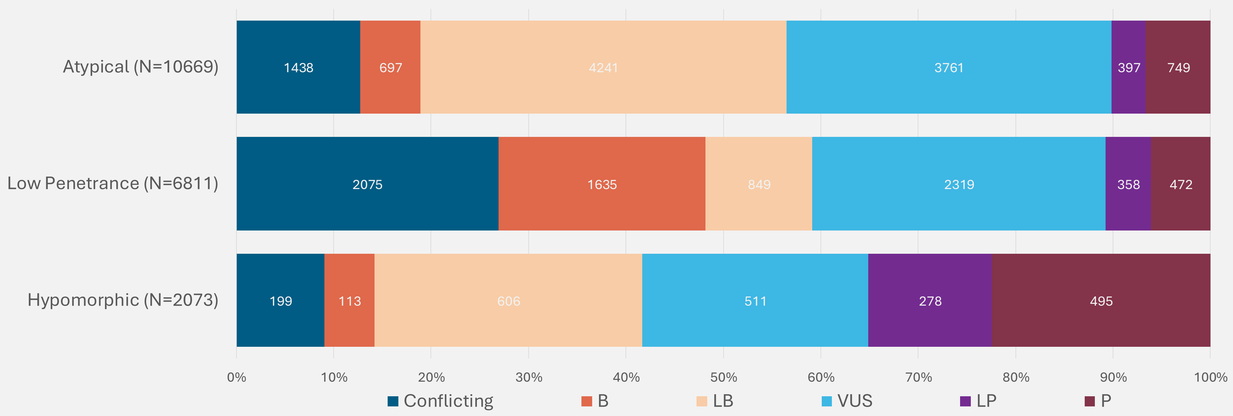

Figure 1 – Clinvar submissions describing Hypomorphic (bottom), Pathogenic Low Penetrance (center) and Atypical risk (top). These terms appeared in submissions for all classification levels and variant types.

TAKE HOME POINTS

- Classification of ARVs presents a challenge in both oncology and rare disease testing.
- ARVs are inconsistently described, classified and/or cited in ClinVar.
- There is limited guidance around best practices for identifying, interpreting, terming and reporting these variant types. Hypomorphic terminology should be used for variants with supporting experimental evidence. Pathogenic Low Penetrance should be used for variants with supporting segregation and family data.
- We suggest "reduced risk Pathogenic" as a preferred overall term for atypical risk variants.
- Unified terminology is critical for consistent variant classifications across laboratories.

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