CLASSIVUS: A Clinical Platform for Reclassifying Uncertain Variants in Hereditary Breast and Ovarian Cancer in a Brazilian Cohort

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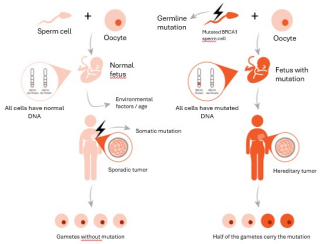
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Abstract

Background: Variants of uncertain significance (VUS) represent a major challenge in clinical genetics, particularly in the context of hereditary breast and ovarian cancer (HBOC). The underrepresentation of Latin American populations in global genomic databases contributes to inconclusive results, hindering accurate genetic counseling and risk-reduction strategies. **Objective:** To reclassify VUS identified in patients with suspected HBOC through a structured application of ACMG/AMP guidelines, integrating computational predictions, population data, and clinical context; and to develop an institutional database (CLASSIVUS) to register, standardize, and track variant classifications within a Brazilian clinical genetics center. **Methods:** A total of 105 patients meeting NCCN criteria for HBOC underwent multigene panel testing. Variants were evaluated according to ACMG/AMP criteria using in silico predictors (PolyPhen-2, SIFT, MutationTaster), population databases (gnomAD, ABraOM), and clinical annotation platforms (ClinVar, Franklin, VarSome, Genoox). Each variant was scored, and a final classification was assigned using CLASSIVUS. **Results:** Thirteen VUS were identified in ten patients across seven genes (ATM, BRCA1, BRCA2, BRIP1, BARD1, CHEK2, and TP53). Seven variants (54%) were reclassified as benign or likely benign, while six (46%) remained VUS. CLASSIVUS enabled centralized, traceable, and reproducible documentation of variant classifications. **Conclusion**: CLASSIVUS is a scalable and integrative tool that enhances evidence-based variant interpretation, supports analytical autonomy, and strengthens the implementation of precision oncology in Brazil.

Introduction

Figure 1. Comparative diagram between sporadic and hereditary tumors. On the left, the somatic mutation is acquired and not inherited. On the right, the germline mutation (e.g., BRCA1) is present from conception and can be transmitted to offspring.



Hereditary breast and ovarian cancer (HBOC) syndrome is primarily associated with pathogenic variants in the BRCA1 and BRCA2 genes. The National Comprehensive Cancer Network (NCCN) guidelines provide recommendations for identifying patients eligible for genetic testing.

Figure 2. Variant classification categories according to ACMG/AMP (2015) criteria.

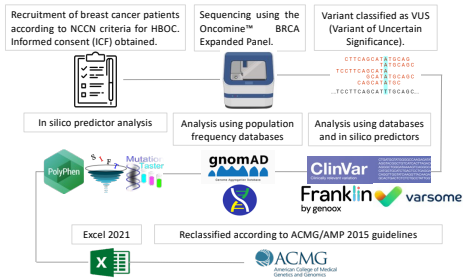


Table 1. ACMG/AMP criteria for genetic variant classification, according to data type and strength of evidence. Source: Adapted from Richards et al. (2015).

Level of Evidence	Criterion	Description
Very Strong	PVS1	Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, or single/multiexon deletion) in a gene where loss of function is a known mechanism of disease.
Strong	PS1	Same amino acid change as a previously established pathogenic variant.
	PS2	De novo variant (both maternity and paternity confirmed) in a patient with no family history of the disease.
	PS3	Well-established in vitro or in vivo studies show a damaging effect on the gene or gene product.
	PS4	The prevalence of the variant in affected individuals is significantly increased compared with controls.
Moderate	PM1	Located in a mutational hot spot and/or critical and well-established functional domain.
	PM2	Absent from population databases (e.g., gnomAD, ABraOM).
	PM3	Detected in trans with a pathogenic variant for recessive disorders.
	PM4	Protein length changes due to in-frame deletions/insertions or stop-loss variants.
	PM5	Novel missense change at an amino acid residue where a different missense change has been determined to be pathogenic.
Strong Benign Evidence	BA1	Allele frequency is greater than 5% in population databases.
	BS1	Allele frequency is greater than expected for the disorder.
	BS3	Functional studies show no deleterious effect on gene or gene product.

Methods and Materials

Figure 01. Workflow diagram. Created by the author in BioRender (2025).



Results

A total of thirteen variants of uncertain significance (VUS) were identified across seven genes: ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, and TP53, in ten patients meeting NCCN criteria for hereditary breast and ovarian cancer (HBOC). Each variant was systematically evaluated according to the ACMG/AMP (2015) guidelines, integrating in silico predictors, population frequency databases, and clinical annotation tools.

After applying the scoring criteria, five variants (38.5%) were reclassified as likely benign (PB), two variants (15.5%) as benign (B), and six (46%) remained VUS due to insufficient or conflicting evidence.

Benign and likely benign classifications were mainly supported by the absence of deleterious functional impact (BS3), conservative amino acid substitution patterns (BP1, BP3, BP4), and high allelic frequencies in population databases such as gnomAD and ABraOM.

Variants that remained uncertain showed moderate evidence of potential pathogenicity (PM1–PM5, PP3–PP5) but lacked strong functional validation. The implementation of the CLASSIVUS database allowed standardized documentation, scoring traceability, and reproducible classification workflows within the institutional context.

Table 2. Classification of VUS by different predictors and their frequency in population databases.

Gene	dbSNP	Variant	ClinVar		Franklin by Genoox		Varsome		Frequency in gnomAD	Frequency in ABraOM
ATM	rs751169467	c.4591A>G	VUS	VUS	AUSENTE	LB	BP1-BP3-BP4-PM2	0.000003721	0.000427	
	rs141687784	c.4148C>T	VUS	VUS	PP3	VUS	BP1-PM2-PP3	0.00004835	Absent	
	rs201958469	c.8560C>T	VUS	VUS	PP3-BS1	VUS	PM2-BP6-PM1-PP3	0.0001896	0.000427	
BARD1	rs864422716	c.2117A>G	VUS	VUS	BP4-PM2	LB	BP1-PM2-BP4	0.000001659	Absent	
BRCA1	rs80358341	c.5425_5430delGTTGTG	VUS	VUS	PM2-PM4	VUS	PM2-PM4	AUSENTE	Absent	
	rs55854959	c.742G>A	VUS	VUS	BP6-PM2	LB	BP1-BP3-BP6-PM2-BP4	0.00003594	Absent	
BRCA2	rs786204274	c.8153>A	VUS	VUS	BP6-PM2	LB	BP1-BP3-BP4-BP6-PM2	0.000006857	0.000854	
	rs431825310	c.4183G>T	VUS	VUS	BP4-PM2	LB	BP1-PM2-BP4	0.000001247	0.000427	
BRIP1	rs200272866	c.4157>G	VUS	VUS	BP4-PM2	LB	BP1-BP4-PM2	0.00006447	0.000427	
	rs542896396	c.3651G>T	VUS	PB	PM2-BP4-BP6	LB	PM2-BP1-BP3-BP4	0.000007974	0.004697	
	rs587780068	c.3412G>T	VUS	VUS	PM2	VUS	PM2-BP1	0.00003199	Absent	
CHEK2	rs375130261	c.1141A>G	VUS	VUS	PM2	LB	BP1-BS3-PM2	AUSENTE	Absent	
TP53	rs587780068	c.472C>T	VUS	LP	BS3-PM1-PM2- PM5-PP2-PP5	P	PM1-PM2-PM5-PP3	0.000007959	Absent	

Legend: Frequency of variants of uncertain significance (VUS) in international and Brazilian genomic databases. Data were collected from the ClinVar, Franklin by Genoox, VarSome, gnomAD, and ABraOM platforms to assess the prevalence of variants across different populations. Absent – No Data; P – Pathogenic; LP – Likely Pathogenic; VUS – Variant of Uncertain Significance; LB – Likely Benign; B – Benign.

Table 3. Reclassification of variants of uncertain significance (VUS) based on ACMG criteria.

Gene	Variant	Applied Criteria	Total Score	Final Classification
ATM	c.4591A>G	BP1 (–2) + BP3 (–2) + BP4 (–2) + PM2 (+2)	–4	LB
	c.4148C>T	BP1 (–2) + PM2 (+2) + PP3 (+1)	+1	VUS
	c.8560C>T	PM2 (+2) + BP6 (–2) + PM1 (+2) + PP3 (+1)	+3	VUS
BARD1	c.2117A>G	BP1 (–2) + PM2 (+2) + BP4 (–2)	–2	LB
BRCA1	c.5425_5430delGTTGTG	PM2 (+2) + PM4 (+2)	+4	VUS
BRCA2	c.742G>A	BP1 (–2) + BP3 (–2) + BP6 (–2) + PM2 (+2) + BP4 (–2)	–6	B
	c.8153>A	BP1 (–2) + BP3 (–2) + BP4 (–2) + BP6 (–2) + PM2 (+2)	–6	B
	c.4183G>T	BP1 (–2) + PM2 (+2) + BP4 (–2)	–2	LB
BRIP1	c.4157>G	BP1 (–2) + BP4 (–2) + PM2 (+2)	–2	LB
	c.3651G>T	PM2 (+2) + BP1 (–2) + BP3 (–2) + BP4 (–2)	–4	LB
	c.3412G>T	PM2 (+2) + BP1 (–2)	0	VUS
CHEK2	c.1141A>G	BP1 (–2) + BS3 (–4) + PM2 (+2)	–4	LB
TP53	c.472C>T	BS3 (–4) + PM1 (+2) + PM2 (+2) + PM5 (+2) + PP3 (+1) + PP5 (+1)	+4	VUS

Legend: Revised classification of variants of uncertain significance (VUS) according to ACMG criteria, with reclassification as P (Pathogenic), LP (Likely Pathogenic), LB (Likely Benign), or B (Benign), based on in silico analyses and genomic databases.

Discussion

The reclassification results demonstrate the relevance of applying ACMG/AMP guidelines in a structured and reproducible manner within a Brazilian population context. Most variants initially reported as uncertain (VUS) were reclassified as benign or likely benign, highlighting the predominance of non-pathogenic variants and the importance of integrating population-specific data such as ABraOM for accurate interpretation.The persistence of a subset of variants as VUS underscores the need for additional functional and segregation studies to strengthen classification evidence. These findings are consistent with international studies, where a large proportion of VUS are later downgraded to the benign spectrum.The development of CLASSIVUS provided a unified environment for variant documentation, evidence scoring, and traceability, improving analytical transparency and promoting institutional autonomy in variant interpretation. This tool can support future updates of reclassified variants, facilitate data sharing, and contribute to the establishment of a national reference framework for hereditary cancer genetics.

Conclusions

The application of ACMG/AMP guidelines enabled a consistent and reproducible reclassification of VUS in patients with hereditary breast and ovarian cancer. Most variants shifted toward the benign spectrum, highlighting the importance of population-specific data for accurate interpretation.The CLASSIVUS platform proved to be a practical tool for standardized documentation and continuous monitoring of variant classifications, strengthening institutional autonomy and supporting precision oncology in Brazil.

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