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Clinical implications of conflicting variant interpretations in the cancer genetics clinic



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ARTICLE INFO

Article history:

Received 18 October 2022

Received in revised form

3 April 2023

Accepted 5 April 2023

Available online 11 April 2023

Keywords:

Cancer genetics

ClinVar

Conflicting variant interpretation

Genetic counseling

Variant interpretation discrepancy

ABSTRACT

Purpose: The aim of this study was to describe the clinical impact of commercial laboratories issuing conflicting classifications of genetic variants.

Methods: Results from 2000 patients undergoing a multigene hereditary cancer panel by a single laboratory were analyzed. Clinically significant discrepancies between the laboratory-provided test reports and other major commercial laboratories were identified, including differences between pathogenic/likely pathogenic and variant of uncertain significance (VUS) classifications, via review of ClinVar archives. For patients carrying a VUS, clinical documentation was assessed for evidence of provider awareness of the conflict.

Results: Fifty of 975 (5.1%) patients with non-negative results carried a variant with a clinically significant conflict, 19 with a pathogenic/likely pathogenic variant reported in *APC* or *MUTYH*, and 31 with a VUS reported in *CDKN2A*, *CHEK2*, *MLH1*, *MSH2*, *MUTYH*, *RAD51C*, or *TP53*. Only 10 of 28 (36%) patients with a VUS with a clinically significant conflict had a documented discussion by a provider about the conflict. Discrepant counseling strategies were used for different patients with the same variant. Among patients with a *CDKN2A* variant or a monoallelic *MUTYH* variant, providers were significantly more likely to make recommendations based on the laboratory-reported classification.

Conclusion: Our findings highlight the frequency of variant interpretation discrepancies and importance of clinician awareness. Guidance is needed on managing patients with discrepant variants to support accurate risk assessment.

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Introduction

In cancer genetics practice, multiple commercial germline genetic testing laboratories may be used, with each of them providing their own categorization of genetic variants, which can lead to clinicians receiving discrepant classification of genetic variants.¹ Variant classification often determines whether the variant has implications for a patient's clinical care, which may include type and frequency of cancer surveillance strategies, prophylactic surgeries, surgical or medical treatment decisions, and recommendation for a cancer genetics evaluation for a patient's family members.^{2,3} Individuals with pathogenic or likely pathogenic (P/LP) variants in hereditary cancer predisposition genes are often provided management recommendations based on the variant, whereas individuals with benign or likely benign variants or variants of uncertain significance (VUS) are managed based on their personal or family history of cancer.²⁻⁷ Therefore, discrepancies in variant classification by different genetics laboratories can have significant clinical implications.

ClinVar is a publicly available online database of variant interpretations, which was created in 2013 with the goal of sharing evidence about variant pathogenicity and establishing consensus interpretations.⁸ Numerous major commercial and research laboratories, expert panels, and other organizations contribute to ClinVar, and clinicians use it as a tool to evaluate variant classifications.

Genetic testing laboratories largely adhere to the joint American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) guidelines for classifying variants, which incorporate evidence such as clinical, population, computational, functional, and segregation data.¹ Nevertheless, discrepancies in variant classification have been identified in 12% to 83% of variants,⁹⁻¹⁵ with the rate depending on the year of the published study, date the variants were last evaluated, specific genes evaluated, and types of laboratories and ClinVar submissions included in the study.^{14,16} Clinically significant discrepancies between the laboratory report and ClinVar, that is, discrepancies between P/LP and VUS, have been reported in 11% of patients with germline findings in cancer susceptibility genes.¹⁰

Despite the adoption of the ACMG/AMP guidelines, discrepant classifications among laboratories remains an issue because of factors including the subjectivity of determining when ACMG criteria are met, laboratory-specific classification schemes, and differences in each laboratory's internal clinical data from patients tested at that particular laboratory.^{9,10,13-15,17,18} Rarer variants and those in lower penetrance genes are predicted to take a longer time to correctly classify, as larger sample sizes are needed.¹⁹ For example, well-studied high penetrance genes such as *BRCA1* and *BRCA2* have a lower reported frequency of variant interpretation discrepancies than other high- and moderate penetrance hereditary cancer genes.²⁰

Additionally, very few (0.1%) discrepancies in *BRCA1* and *BRCA2* involve opposite classifications (P/LP and benign/likely benign).¹⁵

Ninety-six percent of cancer genetic counselors report encountering a variant classification discrepancy, and 99% have concerns about counseling patients with these variants.²¹ Although the presence of clinically significant conflicting variant classification has been established, limited research has assessed the frequency of conflict solely among major commercial clinical laboratories and the impact on patient care. Additionally, the prevalence of conflicting interpretations has not been studied in a defined population tested through a single laboratory. This study aims to describe the clinical impact of conflicting variant interpretations by quantifying the proportion of patients tested through a single commercial laboratory who were found to have a variant with a clinically significant discrepancy, assessing genetics providers' awareness of conflicts, and comparing management recommendations provided to patients with discrepant classifications of the same variant within the same clinical practice.

It is likely that the broad community of cancer genetics practitioners has difficulty interpreting, integrating, and incorporating discordant results into clinical counseling, and this practice is likely to lead to discrepant clinical recommendations given to patients with the same variant. We hypothesize that recommendations for cancer surveillance and genetic testing of family members are likely to correspond with the classification of the variant on the report even when conflicts exist between laboratories.

Materials and Methods

Ascertainment of conflicting variant interpretation prevalence

All reported research was approved by institutional review boards of the participating centers. A cohort of 2000 patients was recruited as part of a multicenter, prospective cohort study of germline panel testing, which has been described previously in detail.²² Individuals were invited to enroll between July 2014 and November 2016 during their genetic counseling appointment at 3 academic centers, USC Norris Comprehensive Cancer Center and Hospital (USC Norris), the Los Angeles County + USC Medical Center (LAC + USC), and Stanford University Cancer Institute. Written informed consent was obtained. All individuals underwent pretest counseling with a board-certified genetic counselor (CGC) or an advanced practice nurse in genetics (APNG), and 688 patients (34%) also met with a medical oncologist or gastroenterologist specializing in cancer genetics. Testing was performed with a 25- or 28-gene panel (Myriad Genetic Laboratories, Inc), which included the following: *APC*, *ATM*, *BARD1*, *BMPRIA*, *BRCA1*, *BRCA2*, *BRIPI1*, *CDHI*, *CDK4*, *CDKN2A* (p14ARF and p16INK4a), *CHEK2*,

EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, and TP53. In July 2016, *GREM1, POLD1, and POLE* were added. After genetic test results were disclosed, medical management recommendations and recommendations for family member testing were recorded in a case report form (CRF). Many variants were reclassified over time; however, the aim of our study was to evaluate interpretation discrepancies at the time of the original test result disclosure. Data were stored in a Progeny database (Progeny Software, LLC).

For all variants identified in individual laboratory-provided reports in the cohort (1326 total variant calls in 975 patients), ClinVar data were analyzed to identify whether there was a clinically significant discrepancy between the classification on the original report and the overall classification in ClinVar. For each variant, the overall ClinVar classification and all submissions to ClinVar before the patient's test report date were recorded. We used application programming interfaces (APIs) E-utilities and Entrez Direct to access and retrieve ClinVar data on November 19, 2019. ClinVar records were manually retrieved for variants that could not be accessed with the APIs. A clinically significant discrepancy ("discrepancy") was defined as a discrepancy between a clinically non-actionable classification (benign, likely benign, or VUS) and a clinically actionable classification (P/LP) between the patient's test report and ClinVar. If the overall ClinVar classification was "conflicting interpretations of pathogenicity," the breakdown of classifications was reviewed to determine whether at least 1 entry had a clinically significant discrepancy from the report classification.

We manually reviewed the available ClinVar entries, as well as ClinVar archives from the month of the patient's report. Each ClinVar archive XML file was searched to identify all records for the particular variant. The record submissions were then evaluated to determine whether there was a discrepancy at that time by a major commercial laboratory, defined as a commercial laboratory in the United States that is Clinical Laboratory Improvement Amendments certified and College of American Pathologists accredited and has at least 1000 submissions to ClinVar. Examples of major commercial laboratories include Ambry Genetics, Color, Counsyl, Fulgent Genetics, GeneDx, Invitae, Prevention Genetics, Quest Diagnostics, and University of Washington. Additionally, submissions from ClinVar-determined expert panels were included. Submissions from research laboratories, GeneReviews, OMIM, and other laboratories were not included.

If there was no conflict in ClinVar during the month of the patient's report, ClinVar archives for 12 months after the date of the laboratory-provided report were reviewed to search for any discrepancies pending ClinVar submissions. All patients found to have a conflict at the time of their report date through the methods described were combined into a single data set.

Evaluation of records for clinical suspicion of VUS pathogenicity

Of the patients with discrepant variants, those in whom the variant was classified as a VUS on the report were evaluated to determine if their genetics provider(s) had knowledge of other laboratories' classification of P/LP or suspicion of pathogenicity. This was assessed by reviewing the CRF and patient medical records. For 3 patients, the CRF was missing and the clinic note was unavailable; these patients were excluded. Provider interpretations and recommendations were evaluated in the context of patient and family history to determine if there was evidence of a provider's suspicion of pathogenicity. Criteria ([Supplemental Table 1](#)) were created based on standard National Comprehensive Cancer Network (NCCN) guidelines^{2,3} and well-established cancer risks associated with specific genes. For example, evidence of a provider's suspicion of pathogenicity included written documentation in the medical record or CRF of another laboratory's classification as P/LP, use of the word "suspicious," or providing screening or risk reduction recommendations according to NCCN guidelines for P/LP variants in the respective gene in the absence of a significant family history that would warrant such recommendations. Examples that were considered to not demonstrate provider suspicion of pathogenicity included not recommending screening beyond general population guidelines when there were established cancer risks and NCCN guidelines for P/LP variants in the respective gene and not recommending genetic testing of family members for the variant. The complete list of general and gene-specific criteria used is detailed in [Supplemental Table 1](#).

Comparison of counseling strategy in discrepant interpretations of the same variants

To compare patients with discrepant classifications of variants between different laboratories, we queried the Cancer Genetics Registry at USC Norris and LAC + USC, where participants underwent multigene panel testing through a variety of commercial laboratories between April 2013 and September 2019. All unique variants within the original cohort of 2000 patients that were identified to have a discrepancy per the methods described above were queried in the Registry database. Four variants in *CDKN2A, CHEK2, and MUTYH* were identified with discrepancies such that another laboratory categorized the variant differently. All patients with these 4 variants who were seen either at the USC Norris or LAC + USC were combined into a single data set, which included 57 patients. For USC Norris and LAC + USC patients, recommendations are likely to be consistent among different providers because of a weekly case conference attended by 3 genetic counselors, a genetics nurse practitioner, and 3 genetics physicians for clinical practice discussions. Recommendations provided for

Table 1 Characteristics of 2,000 study participants

	N	%
Gender		
Female	1614	80.7%
Male	386	19.3%
Age ^a		
<30	100	5.0%
30-39	283	14.2%
40-49	533	26.7%
50-59	503	25.2%
60-69	411	20.5%
70-79	139	7.0%
>79	31	1.6%
Race/Ethnicity		
Non-Hispanic White	811	40.6%
Hispanic White	779	39.0%
Asian	234	11.7%
Unknown/More than one	91	4.6%
Black or African American ^b	75	3.8%
American Indian/Alaska Native	5	0.3%
Native Hawaiian/Pacific Islander	5	0.3%
Cancer Status		
Affected	1442	72.1%
Unaffected	558	27.9%
Original Overall Result		
Positive (with or without a VUS)	243	12.2%
VUS	732	36.6%
Negative	1025	51.2%
Any VUS Identified		
Yes	810	40.5%
No	1190	59.5%

^aMean = 51.5. Std. Dev. = 13.378.

^bThree individuals identified as Black Hispanic.

medical management and genetic testing of family members were assessed through review of CRF and clinical documentation of the results disclosure. There were 5 patients for whom no clinical or research documentation of results disclosure was available.

Data analysis

Descriptive statistics and Fisher exact tests were performed using IBM SPSS Statistics software version 26. A *P* value of less than .05 was considered statistically significant. All Fisher exact tests were two sided.

Results

Demographic characteristics of the study population

A total of 2000 participants were recruited for hereditary cancer panel testing. The most frequently self-reported races and ethnicities were non-Hispanic White (40.6%), Hispanic (39.0%), and Asian (11.7%); 6.8% of

participants reported Ashkenazi Jewish ancestry. Most participants (72.1%) were affected with at least 1 primary cancer, and 8.3% had multiple primaries. Genetic test results included 243 (12.2%) positive (with or without an additional VUS), 732 (36.6%) VUS, and 1025 (51%) negative (Table 1).

Prevalence of clinically significant discrepancies

Among the 975 participants with a positive or VUS result, there were a total of 1326 variant calls reported, representing 943 unique variants. Data were retrieved from ClinVar for 1133 variant calls; 81 were found through a manual search; 112 were not reported in ClinVar. Of 1326, 50 (3.8%) variant calls were found to have a discrepancy at the time of the patient's original genetic test report, representing 50 of 2000 (2.5%) patients who underwent the panel, and 50 of 975 (5.1%) unique patients who had at least 1 variant identified (Figure 1). Classifications were captured for each variant call by each major laboratory and expert panel during the month that the patient's original report was issued (Supplemental Table 2).

There were 14 unique variants in which a conflict was identified. *CHEK2* was the most frequently identified gene with a discrepancy (5 unique variants among 17 patients). Discrepancies were also seen in *APC*, *CDKN2A*, *MLH1*, *MSH2*, *MUTYH*, *RAD51C*, and *TP53* (Figure 2). Complete HGVS nomenclature for each variant is provided in Supplemental Table 3 and was validated by VariantValidator.²³ Of note, the testing laboratory used transcript NM_001128425.2 for *MUTYH*. The variants we describe as c.857G>A and c.934-2A>G are also known as c.773G>A and c.850-2A>G, respectively, when using MANE (Matched Annotation from National Center for Biotechnology Information and European Molecular Biology Laboratory–European Bioinformatics Institute) transcript NM_001048174.2.

Of the 50 patients with conflicting variants, 19 individuals (38%) had a P/LP classification by the laboratory-provided reports (for the variants *APC* c.3920T>A p.(I1307K) and *MUTYH* c.934-2A>G), and 31 individuals (62%) had a VUS classification (for the other 12 variants listed in Figure 2). The proportion of the cohort with a discrepancy by race/ethnicity was 4.7% (11 of 234) for Asians, 1.7% (13 of 779) for Hispanics, and 3.1% (25 of 811) for Non-Hispanic Whites. For individuals with Ashkenazi Jewish ancestry, 9.6% (13 of 136) had a discrepancy; 9 were *APC* c.3920T>A p.(I1307K). When excluding *APC* c.3920T>A p.(I1307K), Asians had the highest prevalence of discrepancy, largely attributed to *MUTYH* c.934-2A>G.

For each of the 50 patients with a conflicting variant, the total number of relatives was counted to assess the broader impact of the variant classification, yielding a total of 291 first-degree relatives (215 living) and 790 second-degree relatives.

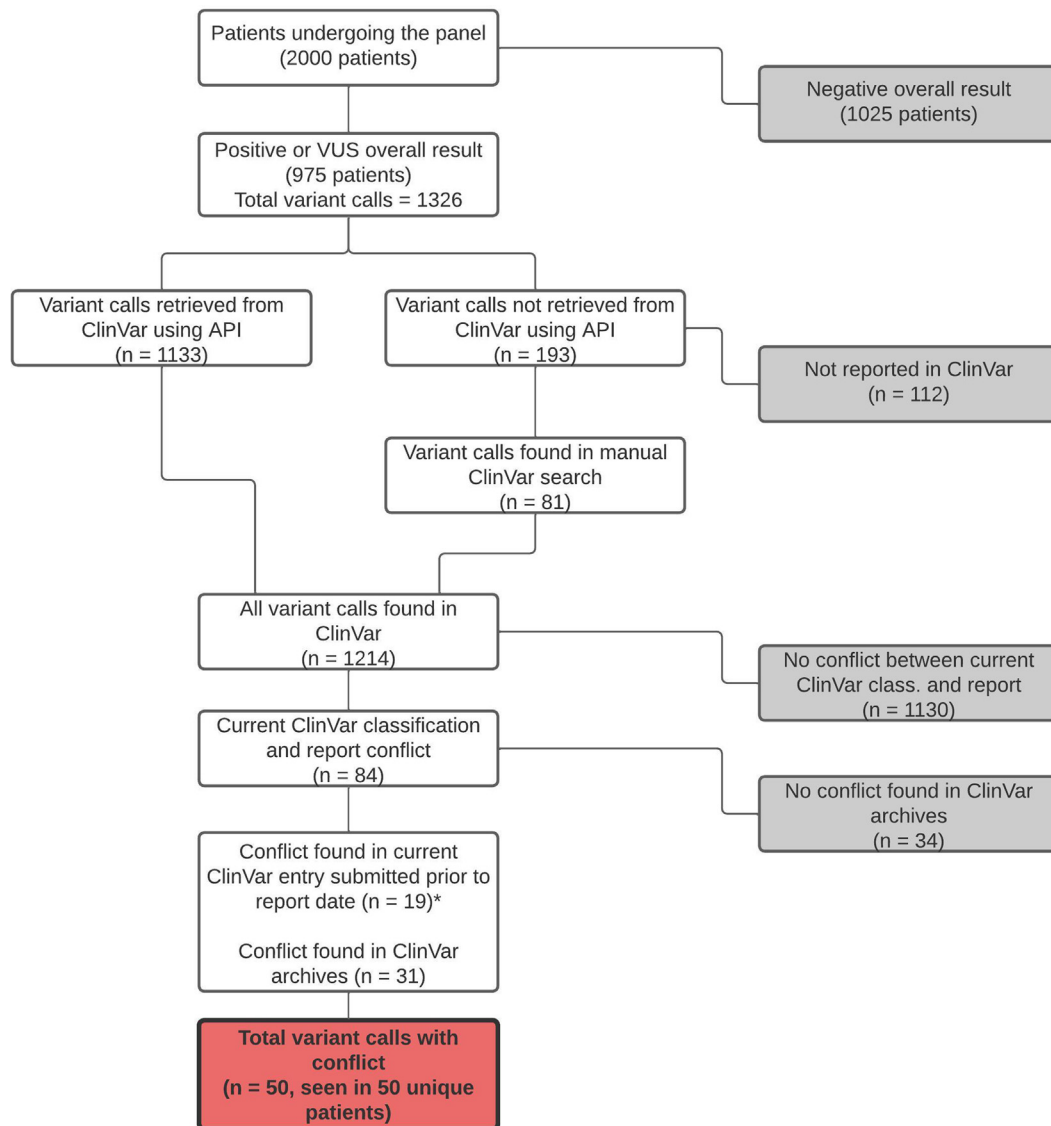


Figure 1 Variant evaluation flowchart. The 1326 variant calls were evaluated individually to determine whether there was a clinically significant conflict among major commercial laboratories at the time of the specific patient's report. Final conflicts are shown in the red box and represent conflicts among major laboratories as outlined by criteria in the Methods. *ClinVar archive search required for the other 65 variants.

Provider suspicion of VUS pathogenicity

Of the 50 patients with a conflicting variant, 31 (62%) had a variant classified as VUS by the laboratory-provided testing reports; 28 had medical records available. Each patient was seen by 1 of 8 genetic counselors or nurse practitioners, and some patients were also seen by 1 of 5 physicians. There was no evidence of provider suspicion of pathogenicity for 64% (18 of 28). The proportion of patients in whom there was provider suspicion varied by specific variant (Figure 3). For high penetrance genes (*CDKN2A*, *MLH1*, and *TP53*), only 1 of 9 patients received counseling that acknowledged the variant discrepancy.

Discrepant classifications of the same variant within a clinical practice

The Cancer Genetics Registry at USC Norris and LAC + USC allowed for identification of additional individuals with the same discrepant variants (*CDKN2A* c.146T>C, *CHEK2* c.349A>G, *CHEK2* c.470T>C, and *MUTYH* c.934-2A>G). There were 57 total patients (including 24 from the original cohort and 33 from the Registry) with these 4 variants. Results for these Registry patients were received between April 2014 and June 2019.

Three of these variants were seen in 3 or more patients and were analyzed further to compare medical management

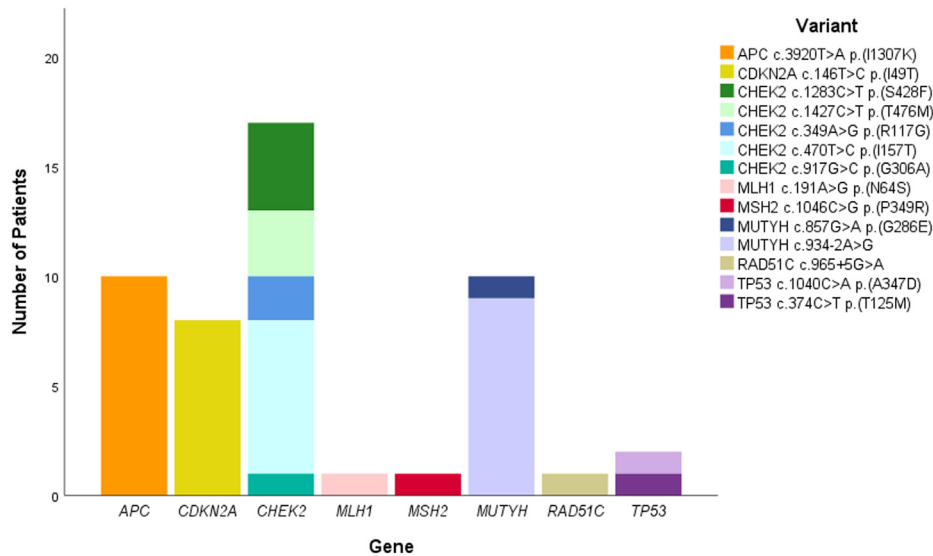


Figure 2 Distribution of variants with clinically significant conflicts. The 50 patients with variants who had clinically significant discrepant classifications are distributed by unique variant and aggregated by gene. The order of variants in the key corresponds to the vertical order of variants in the graph.

recommendations between those with a P/LP classification and a VUS classification (Table 2). Although the sample is small, it revealed that most patients are counseled according to their test report and that personal and family history as well as the patient's current disease status influenced the recommendations. For example, in patients with *CDKN2A* c.146T>C, 2 of the 14 patients with a laboratory-reported VUS classification were recommended to undergo a skin examination, but one had a personal history of melanoma and the other had a family history of melanoma. There was a statistically significant association between report classification of this variant and skin examination recommendation

when excluding those with a personal or family history of melanoma (Fisher exact $P = .018$).

Another example included patients with the *MUTYH* c.934-2A>G variant, including 18 classified as P/LP and 2 as VUS (Table 2). Fourteen of those assessed with a P/LP classification of this variant were recommended to undergo colonoscopy every at least every 5 years, which was the clinical group's recommendation throughout the period of the study for individuals with monoallelic P/LP variants in *MUTYH*. For both individuals with a VUS classification, no colonoscopy recommendations were given. Among patients with this variant, providers were significantly more likely to

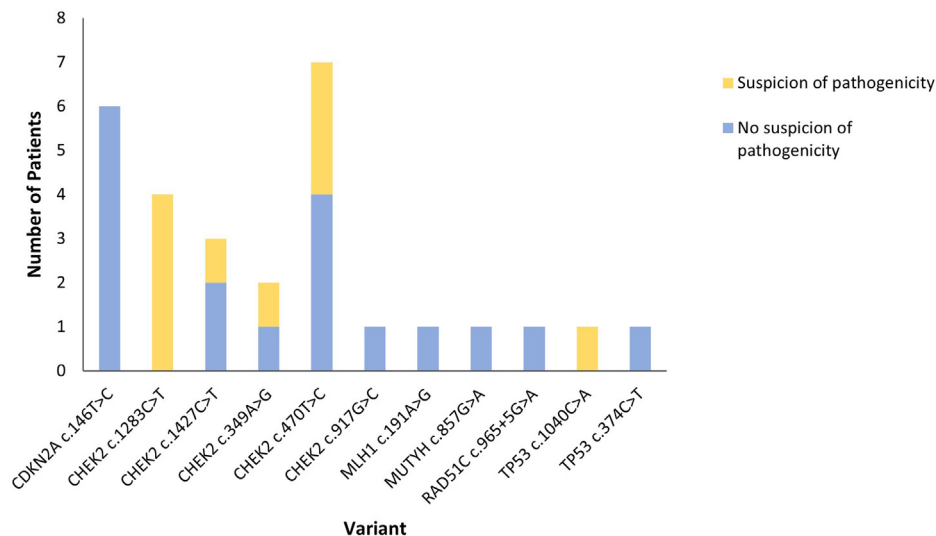


Figure 3 Suspicion of pathogenicity in patients with variants classified as VUS. $N = 28$. The number of patients with each variant for which there was and was not evidence of provider suspicion of pathogenicity is shown. VUS, variant of unknown or uncertain significance.

Table 2 Comparison of medical management recommendations for discrepant variants

	Classification		Fisher's exact <i>P</i> -value
	P/LP	VUS	
	<i>N</i> (%)	<i>N</i> (%)	
<i>CDKN2A</i> c.146T>C ^a			
Skin exam recommended			
No	0 (0%)	9 (81.8%)	0.077 ^c
Yes	2 (100%)	2 (18.2%) ^b	
Total	2	11	
Pancreatic cancer screening recommended			
No	1 (50.0%) ^d	11 (100%)	0.154 ^f
Yes	1 (50.0%) ^e	0 (0%)	
Total	2	11	
Targeted variant testing recommended			
No	1 (50.0%) ^g	10 (90.9%)	0.295
Yes	1 (50.0%)	1 (9.1%) ^h	
Total	2	11	
<i>CHEK2</i> c.470T>C			
Colonoscopy frequency ⁱ			
General population or no recommendation given	2 (22.2%)	3 (60.0%)	0.266
Every 5 years or more frequently	7 (77.8%)	2 (40.0%) ^j	
Total	9	5	
Breast MRI recommended ^k			
No	2 (25.0%)	2 (50.0%)	0.547
Yes	6 (75.0%)	2 (50.0%) ^l	
Total	8	4	
Targeted variant testing recommended ^m			
No	1 (20.0%) ⁿ	5 (83.3%)	0.08
Yes	4 (80.0%)	1 (16.7%) ^o	
Total	5	6	
<i>MUTYH</i> c.934-2A>G			
Colonoscopy frequency ^p			
General population or no recommendation given	1 (6.7%)	2 (100%)	0.022*
Every 5 years or more frequently	14 (93.3%)	0 (0%)	
Total	15	2	
Targeted variant testing or <i>MUTYH</i> sequencing recommended			
No	2 (15.4%)	2 (100%)	0.057
Yes	11 (84.6%)	0 (0%)	
Total	13	2	

P/LP represents a classification of pathogenic/likely pathogenic. Cancer screening recommendations are for individual patients, and targeted variant testing refers to testing of family members for the respective variant. *MUTYH* sequencing refers to sequencing of the gene to assess either for the presence of biallelic P/LP variants, which would result in *MUTYH* associated polyposis syndrome (MAP), or to assess status of the patient's partner to ascertain risk of MAP in offspring. Total N for familial testing represents total unique families.

^aDocumentation of results disclosure was unavailable for three individuals with VUS classifications.

^bOne had a personal history of melanoma, and one had a family history of melanoma.

^cWhen controlling for no personal or family history of melanoma, Fisher's exact *P* = 0.018*.

^dPatient already affected with this disease. Knowledge of conflicting interpretations was not apparent.

^eNo personal or family history of pancreatic cancer. Results disclosure note discussed other laboratories' VUS classification, indicating that provider was aware of conflict. Patient was under recommended age to begin screening, and note discussed the possibility for recommendations to change due to the ambiguity of the variant. Patient recommended to return to clinic in two years for updated management recommendations.

^fWhen controlling for no personal or family history of pancreatic cancer, Fisher's exact *P* = 0.083.

^gProvider displayed awareness of conflict, and this appeared to play a role in not recommending familial testing.

^hVUS tracking studies recommended for family history of melanoma.

ⁱOne deceased patient, one with incomplete data, and three with active metastatic disease were excluded, as cancer screening recommendations were not provided or available.

^jBoth demonstrated provider awareness of conflicting interpretations; no family history of colorectal cancer.

^kOnly females were included.

^lOne also had a pathogenic variant in *ATM* that appeared to drive this recommendation; the other had demonstrated provider awareness of conflicting interpretations.

^mThere were 13 unique families with this variant; one was excluded because the patient was deceased when the results were received, and no recommendations were provided, and the other was excluded because all at-risk family members had already been tested for the variant prior to presenting to Cancer Genetics.

ⁿUnclear why not recommended; not discussed in clinic note. It may be possible that it was recommended but not documented in the note.

^oAppeared to be driven by knowledge of conflict.

^pDocumentation of results disclosure was available for 18 patients. One patient was added because although there was no CRF or original genetics results disclosure note, there was another provider's note available which discussed the colonoscopy recommendations from the genetics provider. One deceased patient and one with incomplete data were excluded.

make colonoscopy recommendations based on the laboratory-reported classification (Fisher exact $P = .022$).

Counseling strategy for discrepant classifications by the same provider

Three genetic providers were involved in counseling patients with discrepant classifications of the same variant. All 3 providers displayed differences in counseling strategy when counseling patients with different classifications of the same variant (Supplemental Table 4A-E). For example, 1 provider saw patients with discrepant classifications of *MUTYH* c.934-2A>G and disclosed their results 1 month apart. Although neither patient had any personal or family history of colon cancer or polyps, enhanced colonoscopy screening and targeted variant testing were recommended for the patient with LP classification and not for the patient with VUS classification. The patient with VUS classification was not counseled with knowledge of the conflict. Neither patient had additional P/LP variants identified on testing. Additional case examples are available in Supplemental Table 4A-E.

Discussion

This study describes the clinical impact of variant interpretation discrepancies between laboratories. Clinically significant conflicts were found in 2.5% (50 of 2000) of the original cohort of patients and 5.1% (50 of 975) of patients with a non-negative result. Conflicts were found in variants in *APC*, *CDKN2A*, *CHEK2*, *MLH1*, *MSH2*, *MUTYH*, *RAD51C*, and *TP53*. Our comparison of recommendations for discrepant variants supports our hypothesis that clinicians are more likely to provide clinical recommendations according to the laboratory-reported classification.

This study builds on prior studies analyzing variant discrepancies. For example, a previous study found that 11% of patients with a variant identified on hereditary cancer panel testing had a clinically significant discrepancy.¹⁰ However, the study included all ClinVar submissions and was not limited to clinical laboratories. In a critique of the study by Balmaña et al, the variants were re-evaluated to only include submissions from clinical laboratories and ClinVar-determined expert panels (excluding literature and research submissions), and only 5.5% of patients had a clinically significant conflict.²⁴ This is consistent with our finding that 5.1% of those with non-negative results had a variant with a clinically significant conflict when their report was issued. By focusing on conflicts that have the potential to affect

medical management and only including ClinVar submissions by laboratories that perform a considerable amount of clinical testing, our findings likely reflect the proportion of patients who may actually be affected by these discrepancies.

The genes and variants identified to have discrepancies are relatively consistent with previously published studies.^{10,14,24} *CHEK2* had the greatest number of unique variants with conflicts and affected the greatest number of patients (5 unique variants among 17 patients). In the study by Balmaña et al,¹⁰ 63.2% (36 of 57) of the variant calls with a clinically significant conflict were in *CHEK2*. Other genes with conflicts in their study included *APC*, *BRIP1*, *CDKN2A*, *FH*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, and *RAD51C*. We similarly identified discrepancies in *APC* (specifically p.(I1307K)), *CDKN2A*, *MUTYH*, and *RAD51C*, and additionally in *MLH1*, *MSH2*, and *TP53*. Similarly, in the study by Harrison et al,¹⁴ *CHEK2* had the greatest number of conflicts of all cancer-related genes.

Variants in *CHEK2*, particularly missense variants, are known to be challenging to classify.^{17,25} Perhaps this is because *CHEK2* is a moderate penetrance gene, its expected phenotype (breast or colon cancer) is common, and the genetics community's understanding of its associated cancer types and specific risk estimates is continuing to evolve.²⁵ Therefore, data from phenotype studies may not be as useful for determining variant classification. Three variants in *CHEK2*—c.470T>C p.(I157T), c.1283C>T p.(S428F), and c.1427C>T p.(T476M)—are known founder variants with conflicting data on pathogenicity.^{19,25} Some laboratories and publications describe these missense variants as low penetrance,²⁵ which are known to have high rates of discordance.¹⁶ All conflicting variants in *CHEK2* identified in our study were missense, with 14 of 17 patients having one of the founder variants.

Low penetrance variants are challenging to classify because they do not fall into any of the categories outlined in the ACMG/AMP guidelines.^{1,16,26} Individuals with *APC* c.3920T>A p.(I1307K) made up 20% (10 of 50) of conflicts. Previous research and national guidelines have determined that this is a low penetrance founder variant that confers a moderately increased risk of colorectal cancer.³ However, this variant is still classified as VUS by several major laboratories. Development of guidelines for classification of low penetrance variants, with criteria similar to the ACMG/AMP guidelines,¹ may be helpful in resolution of some of these conflicts. Furthermore, gene-specific interpretation guidelines will aid in the interpretation of variants in moderate- and low penetrance genes, as the use of gene-specific criteria has been shown to decrease the frequency of discordant interpretations.¹¹

Conflicts can also be prevalent in genes that are typically highly penetrant, such as *TP53*, in which 1 study showed that 11% of families had a variant with a clinically significant discrepancy.²⁷ Both *TP53* variants identified in our study were also identified in the study by Frone et al.²⁷ It is possible that some of the variants with conflicts in high penetrance genes, such as *TP53*, may truly be low- or moderate penetrance variants and may produce an attenuated phenotype compared with other P/LP variants in the gene.

Although the number of patients with discrepancies is relatively small, the clinical impact on these patients can be substantial. NCCN provides guidelines for cancer surveillance and risk reduction in individuals with P/LP variants in cancer predisposition genes.^{2,3} Patients with potentially pathogenic variants may not be recommended the care associated with the variant. Many insurance companies use NCCN guidelines to determine coverage of services²⁸; therefore, services could be denied even if the provider were to recommend the screening based on a known conflict, particularly in individuals who do not meet NCCN criteria for enhanced screening based on family history alone. Additionally, there are now Food and Drug Administration approvals and clinical trials for targeted cancer treatments that use germline or tumor variants to inform treatment and are available to individuals with a P/LP variant in specific genes.^{2,3} Variant interpretation discrepancies could lead to discrepancies in treatment options for patients with the exact same cancer type and germline variant. To resolve discrepancies, we encourage collaboration between laboratories and evaluation of variants by ClinGen-determined expert panels.

Our study revealed that only 10 of 28 (36%) patients with a laboratory-reported VUS were counseled with knowledge of a conflict when their variant was classified as P/LP by another major commercial laboratory. Previous research has shown that most cancer genetic counselors do not evaluate variant evidence beyond the laboratory report for most of their patients, and the primary barrier is lack of time.²⁹ Perhaps another contributing factor is that most VUS are downgraded to benign.³⁰⁻³² Additionally, genetics providers may be less likely to research a VUS in ClinVar when a family history does not fit the respective gene's phenotype. However, results of this analysis show that cancer genetic counselors cross-checking variants in ClinVar could lead to the identification of variant discrepancies in 5% of variants on genetic test reports and may help avoid counselors providing different recommendations to patients with the same variant. Because discrepancies can be critical to patients' clinical management, it could be helpful for professional organizations such as NCCN to provide guidance to providers about evaluating all variants in ClinVar before post-test counseling. This also highlights the importance of clinical laboratories submitting classifications to ClinVar; we encourage professional organizations to consider incorporating this recommendation into practice guidelines.

Awareness of variant conflicts is likely even lower among nongenetics professionals. Nongenetics oncology providers have displayed limited understanding of VUS³¹ and are thus more likely to misinterpret results. Nongenetics providers may have lower volume of genetic testing and may be less familiar with recurrent variants or how to handle variant reclassifications. Therefore, the results of our analysis may be even more pronounced among nongenetics providers.

Our review of case examples demonstrated that counseling is challenging even when a provider is aware of a conflict, and recommendations did not always completely align with a VUS or P/LP classification. Clinical genetic counselors are becoming increasingly involved in variant interpretation in determining how to appropriately manage their patients.^{12,29,32} When genetic counselors are aware of a laboratory conflict or have their own conflicting interpretation based on available evidence, they report discussing this with the laboratory, medical team/colleagues, and patient/family.²⁹ Genetic counselors and medical geneticists have reported most often following the laboratory's classification, but occasionally managing the patient based on their own interpretation of the variant; for VUS that the clinician suspects is pathogenic, this sometimes includes a recommendation for screening tests but not invasive procedures.³² Genetic counselor continuing education and professional organization practice guidelines on how to counsel patients with discrepant variants are desired by genetic counselors²¹ and would provide awareness and guidance on this issue.

A limitation of our study is that the initial cohort from the longitudinal cohort study was tested in 2014-2016. Patients tested today by an experienced provider may be more likely to receive counseling with knowledge of the conflicts. Additionally, some of the variants we describe have since been reclassified. However, given the increase in identification of VUS over time as multigene panels become increasingly larger and more widely used in unselected populations,³³ conflicts are likely to remain prevalent, and the principles we describe in this paper will continue to affect patient outcomes in clinical practice.

Another limitation of this study is the small chance that variants with clinically significant conflicts were missed by the analysis of ClinVar archives because of variants not being reported at all by a laboratory or laboratories not submitting updates on time. Additionally, when assessing management recommendations and counseling strategy with discrepant classifications, generalizability is limited because the sample size was small and all patients included in this part of the analysis were seen through 1 institution. This study was not able to identify whether certain racial/ethnic populations are more likely to have variants with discrepancies.

In conclusion, the findings from this study support previously published literature finding that approximately 5% of patients with non-negative results on hereditary cancer panel testing are found to have a variant with clinically significant discrepancies among major commercial laboratories. This study described provider awareness of clinically

significant conflicts when counseling patients with a VUS classified as P/LP by other laboratories and found that a minority of patients seemed to be counseled with provider awareness of the conflict. A detailed case analysis identified discrepant counseling strategies used for different patients with the same variant within the same institution and even by the same genetics provider. Our findings provide evidence that variant interpretation discrepancies can have profound clinical implications, highlighting the importance of clinician awareness and the need for guidance on managing patients with discrepant results.

Data Availability

Data are available upon request to the corresponding author.

Funding

This study is supported by Myriad Genetics and National Institutes of Health (Grant No. KL2-TR000131).

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Ethics Declaration

This work was institutional review board reviewed and approved by USC, UCI, and Stanford institutional review boards. Written informed consent was obtained from all patients.

Conflict of Interest

Gregory E. Idos reports research funding from Myriad Genetics (Inst). Jason A. Zell serves in a consulting role for Tempus. Charité N. Ricker reports research funding from Myriad Genetics (Inst). Kevin J. McDonnell has served in a consulting or advisory role for Brogent International and

reports research funding from Myriad Genetics. Uri Lada- baum serves as an advisor for Universal Dx, Lean Medical, Kohler Ventures, Vivante, and a consultant for Freenome, Guardant, Medtronic, Neptune Medical, Medial EarlySign. Stephen B. Gruber reports that he is co-founder with equity with Brogent International. Giovanni Parimigiani reports that he holds equity as a co-founder in Phaeno Biotechnologies, is on the SAB of Realm Idx (which owns Ambry Genetics) and currently consults for Delphi Diagnostics and (pro bono) for Martingale Labs. He also co-leads the BayesMendel laboratory, which licenses the BayesMendel package which include several ML tools for the computation of carrier probability of cancer susceptibility genes and future cancer risk. He does not derive any personal income from these licenses. Danielle Braun co-leads the BayesMendel lab, which develops and maintains the BayesMendel software package. This includes a variety of risk assessment tools, including BRCAPRO, PancPRO, MelaPRO, MMRpro and is licensed for commercial use. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (<https://doi.org/10.1016/j.gim.2023.100837>) contains supplementary material, which is available to authorized users.

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