Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing

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ABSTRACT

Purpose
Massively parallel sequencing allows simultaneous testing of multiple genes associated with cancer susceptibility. Guidelines are available for variant classification; however, interpretation of these guidelines by laboratories and providers may differ and lead to conflicting reporting and, potentially, to inappropriate medical management. We describe conflicting variant interpretations between Clinical Laboratory Improvement Amendments–approved commercial clinical laboratories, as reported to the Prospective Registry of Multiplex Testing (PROMPT), an online genetic registry.

Methods
Clinical data and genetic testing results were gathered from 1,191 individuals tested for inherited cancer susceptibility and self-enrolled in PROMPT between September 2014 and October 2015. Overall, 518 participants (603 genetic variants) had a result interpreted by more than one laboratory, including at least one submitted to ClinVar, and these were used as the final cohort for the current analysis.

Results
Of the 603 variants, 221 (37%) were classified as a variant of uncertain significance (VUS), 191 (32%) as pathogenic, and 34 (6%) as benign. The interpretation differed among reporting laboratories for 155 (26%).

Conclusions
Conflicting interpretation of genetic findings from multiplex panel testing used in clinical practice is frequent and may have implications for medical management decisions.

INTRODUCTION

Advances in next-generation sequencing technology have led to the development of multiplex panel testing for the molecular diagnosis of inherited cancer susceptibility. Commercially available panels differ in their exact composition but usually include moderate-penetrance and high-penetrance genes (with mutations reported in the literature to be associated with a relative risk [RR] of cancer between 2 and 5, or greater than 5, respectively). Genes in which mutations are associated with susceptibility to inherited cancer have been rapidly incorporated into these panels, often before robust evidence of the magnitude of the association is known. For some genes, the relatively low prevalence of mutations makes it difficult to obtain reliable estimates of the associated cancer risk.

Variants of uncertain significance are frequent in panel testing and can be challenging to resolve. Despite the availability of public databases for sharing genetic variants, the development of prediction models based on protein structure and function, and the potential for laboratory-based functional analyses to determine the pathogenicity of some variants, discordant interpretation of the clinical pathogenicity of variants remains a frequent problem. Different standardized classification systems for interpretation of sequence-based results have been developed. The ClinVar database is a publicly available database that has allowed clinical laboratories to submit their identified variants and

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share their interpretation. All accessions of the same genetic variant provided by different submitters are maintained in ClinVar, which allows tracking of the changes and sharing of the evidence used for interpretation. However, submission to ClinVar is voluntary and not all laboratories choose to submit data. Moreover, resolution of the diverse submissions is a voluntary activity for experts and a Herculean task. From a clinical perspective, it is relevant to quantitate the frequencies and describe the patterns of variants with conflicting interpretations, particularly those that may impact medical management. Unrelated individuals or even members from the same family with the same genetic variant tested by different clinical laboratories may be given a different clinical assessment of variant pathogenicity. The ordering provider may not be aware that a different laboratory testing a patient’s relative has provided a different interpretation. It is crucial to identify variants with conflicting interpretations among laboratories, the frequencies and types of discrepancies, and the underlying reasons for these discrepancies to enable specific guidance for variant curation and to increase the consistency of variant interpretation among the laboratories.

Here, we describe the frequencies and types of genetic findings with conflicting interpretations in non-BRCA genes tested as part of panels assembled by Clinical Laboratory Improvement Amendments–approved commercial laboratories. Data were collected from individuals who underwent clinical testing and enrolled in a prospective registry. This registry includes patients with results from some commercial laboratories that currently do not deposit data in ClinVar.

RESULTS

Overall, 518 participants enrolled into PROMPT were considered eligible for this analysis. Their median age was 52 years (range, 44-61 years) and 95% were female. Overall, 427 (82%) were white, and 350 (68%) had a cancer diagnosis, mostly breast cancer (n = 188; 36%). Thirty-one percent had multiple primary tumors. A total of 419 participants (81%) reported being invited into PROMPT by their health-care provider or by the laboratory where the testing had been performed (Table 1).

These 518 participants reported 603 genetic variants with multiple interpretations by several commercial laboratories and/or submissions to ClinVar. Of the 518 participants included in this analysis, 165 provided information from testing done in a laboratory that does not submit to ClinVar. The most frequent gene with sequence alterations reported through PROMPT was CHEK2 (n = 117), followed by ATM (n = 105) (Fig 2).

Regarding the type of results according to their clinical interpretation in ClinVar, 220 (36%) were consistently classified as VUS, 191 (32%) as pathogenic/likely pathogenic, and 34 (6%) as benign/likely benign, while 155 (26%) were classified as conflicting interpretation. Among these 155, 26% of them were in CHEK2; 20% in ATM; 8% in RAD51C; 7% in PALB2; 5% in BARD1; 4% in NBN and APC; 3% in RAD50, PMS2, TP53, and MUTYH; 2% in BRIP1 and FANCC; and the remainder were distributed among the other genes at approximately 1% each (Fig 3). Of the 155 discordant findings, 56 (36%) were reported as pathogenic/likely pathogenic by at least one laboratory but not by all laboratories (ie, clinically significant; Table 2).

Of 117 findings with multiple interpretations for CHEK2, 41 (35%) were conflicting, and the majority (n = 36; 88%) would be characterized as clinically significant. Eighteen were c.470T>C (p.Ile157T) variant and 12 were c.1283C>T (p.Ser428Phe), both classified as either pathogenic/likely pathogenic or as VUS (Table 3). Other variants in this gene with a conflicting
interpretation between pathogenic/likely pathogenic or VUS are described in Table 2. In addition, 32 of 105 variants (30%) in the ATM gene with multiple submissions were classified as conflicting. However, all of these ATM variants ranged from benign/likely benign or VUS (Appendix Fig A1, online only; Table 2) and, therefore, should not alter medical management. In PALB2, nine of 49 findings (18%) reported with multiple submissions were conflicting; one, c.3113G>A(p.Trp1038Ter), which was reported three times, was classified as either pathogenic or VUS, and the remainder were interpreted as either benign or VUS. In BRIP1, three of 33 findings (9%) were discordant; one of them was reported twice [c.139C>G(p.Pro47Ala)] and was classified as either likely pathogenic or VUS. In RAD51C, 13 of 26 (50%) findings (50%) were conflicting. Twelve of these findings corresponded to the variant c.790G>A (p.Gly264Ser), which was submitted as either benign or VUS, and one, the c.1026+5_1026+7delGTA, was interpreted as both likely pathogenic and VUS. In the NBN, six of 20 findings (30%) reported were conflicting. Two of them, c.511A>G(p.Ile171Val) and c.643C>T(p.Arg215Trp), were reported twice each, and had a two-step difference in conflicting interpretation in ClinVar (pathogenic, VUS, benign/likely benign). Other genes with conflicting interpretation are listed in Table 2.

**DISCUSSION**

One quarter of the clinical genetic results from commercially available multiplex cancer panels and reported at the PROMPT registry had conflicting interpretations within ClinVar. Most of the variants with conflicting interpretations were in CHEK2, followed by ATM, RAD51C, and PALB2. Many conflicting interpretations are of low clinical significance because the discrepancy ranged between an interpretation of benign/likely benign or VUS; therefore, medical management should default to personal and family history. However, the identification of a VUS can cause a great deal of uncertainty for patients and providers alike and increase the risk for inappropriate medical management. For example, it is inappropriate to recommend oophorectomy based on a VUS finding alone. Of greater concern, 36% of conflicting results appeared to be clinically relevant, because they were either reported as pathogenic/likely pathogenic or as a VUS by different clinical laboratories. In this regard, CHEK2, PALB2, and BRIP1 were most frequently identified as having discordant interpretation between these two levels of pathogenicity. As these genes are being incorporated into clinical practice as part of cancer risk assessment, tailored screening and cancer prevention recommendations, or within tumor panel sequencing for potential targeted therapy, it will be critical to standardize their curation and clinical classification of variants to provide appropriate management for mutation carriers and their families.

A strength of this study is the inclusion of individuals who underwent testing at laboratories that do not submit data to ClinVar. Our data highlight several specific variants of interest related to differential reporting. Of 117 of the CHEK2 findings reported in PROMPT to date, 41 (35%) were conflicting. The c.470T>C(p.Ile157Thr, I157K) and the c.1283C>T(p.Ser428Phe, S428F) variants were the most common with conflicting interpretation between pathogenic/likely pathogenic and VUS. The putative pathogenicity of I157T has long been studied. Several
of clinical interpretations from VUS to pathogenic (low penetrance), likely pathogenic, and pathogenic (Table 3).

Differences in interpretation of the evidence for the pathogenicity of CHEK2 S428F are less pronounced but still lead to conflicting reports of pathogenic, pathogenic/low penetrance, and VUS. All laboratories agree that the S428F variant is located in a well-established functional domain16; and all but one agree that there is evidence of an increased prevalence in affected individuals3 controls. However, three laboratories report that functional studies are supportive of the damaging effect of CHEK2 S428F, and one laboratory concludes that the impairment is variable.

Finally, two laboratories report the literature evidence from cosegregation of the CHEK2 variant with the disease; only one laboratory reports the prediction of pathogenicity by in silico algorithms, and another laboratory emphasizes the contribution of this variant to tumorigenesis by loss of heterozygosity (Table 3).

The examples of the discrepancies involving CHEK2 I157T and S428F also may reflect the challenges of describing a so-called low-penetrance susceptibility allele (RR < 2) in a format designed for high-penetrance alleles.17

Another interesting variant with conflicting interpretation between likely pathogenic and VUS is the c.139C>G substitution (p.Pro47Ala) in the BRIP1 gene, which was observed in two unrelated participants from PROMPT. This variant was first described in 2001,18 when the BRIP1 protein was found to interact with BRCA1 and contribute to its DNA repair function. This variant was initially identified in an individual with early-onset breast cancer and a family history of breast and ovarian cancer; segregation analysis was not available and loss of heterozygosity in the tumor was not demonstrated. Since then, it has been reported in the literature several times as a breast or ovarian cancer susceptibility gene in affected individuals, but has also been observed in control subjects.19,20 Two clinical laboratories providing a summary of their clinical interpretation in ClinVar differ in their assessment of the meaning of low function observed in the experimental studies in regard to cancer susceptibility; one laboratory highlights that it is present in population databases, albeit as a very rare allele (0.04%); and only one endorses the supporting evidence provided by the prediction of in silico algorithms. In phase 2 of the PROMPT project, enrollment of family members will be encouraged and cosegregation analysis of this BRIP1 variant may be undertaken, which may help provide one more piece of supporting evidence to clarify its pathogenicity.

Findings of discordant interpretation of results in genetic testing are not limited to oncologic settings nor ClinVar. Similar discrepancies also have been observed in hereditary connective-tissue disorders and are felt to be due to lack of submission of data to public databases, limited use of allele frequency data, and varying consideration of protein structure and function.21 In the current study, the discrepancies between commercial laboratories in interpretation of variants in cancer-related genes seem to be mostly based on differences in the interpretation of evidence. Efforts have already been initiated among laboratories to resolve these differences.22 The lack of a gold standard test for pathogenicity, or a noncontroversial interpretation of functional analyses, suggests that discrepant interpretation of challenging variants, particularly missense and splice-site variants, will persist for some time.
Multigene testing for cancer susceptibility is a complex endeavor characterized by challenges in curation and reporting of variants; unfortunately, this study demonstrates that conflicting interpretation of those variants may be relatively frequent. The rates of discrepant interpretation reported herein support the need for initiatives focused on harmonizing variant interpretation in the context of shared data. We encourage clinical laboratories to submit their findings to ClinVar and other public databases with...
### Table 2. Genetic Variants With Conflicting Interpretation

| Gene | No. With Multiple Submissions | Total No. (%) | Description of Variants (No. identified) | Type of Variant |
|------|------------------------------|---------------|------------------------------------------|-----------------|
| **APC** | 10 | 4 (40) | c.3920T>A (p.Ile1307Lys) (2) | Missense |
| | | | c.1240C>T (p.Arg414Cys) (2) | Missense |
| **BRIP1** | 33 | 2 (6) | c.139C>G (p.Pro47Ala) (2) | Missense |
| **CDKN2A** | 5 | 2 (40) | c.9-32dup24 (2) (p.Pro11_Ser12insAlaAlaGlySerSerMetGluPro) | Duplication |
| **CHEK2** | 117 | 36 (31) | c.470T>C (p.Ile157Thr) (18) | Missense |
| | | | c.1283C>T (p.Ser428Phe) (12) | Missense |
| | | | c.1111C>T (p.His371Ty)s | Missense |
| | | | c.1427C>T (p.Thr476Met) | Missense |
| | | | c.483_485delAGA (p.Glu161del) | Deletion |
| | | | c.715G>A (p.Glu239lys) | Missense |
| **FH** | 1 | 1 | c.1431_1433dupAAA (p.Lys477_Asn478insLys) | Duplication |
| **MSH6** | 20 | 1 (5) | c.2927G>A (p.Arg976His) | Missense |
| **MUTYH** | 22 | 3 (14) | c.821G>A (p.Arg274Gln) | Missense |
| | | | c.934-2A>G (2) | Splice acceptor |
| **NBN** | 20 | 4 (20) | c.511A>G (p.Ile171Val) (2) | Missense |
| | | | c.643C>T (p.Arg215Trp) (2) | Missense |
| **PALB2** | 49 | 3 (6) | c.3113G>A (p.Trp1038Ter) (3) | Nonsense |
| **RAD51C** | 26 | 1 (4) | c.1026+5_1026+7delGTA | Deletion |

### Between Pathogenic/Likely Pathogenic and Uncertain Significance (n = 57)

| Gene | No. With Multiple Submissions | Total No. (%) | Description of Variants (No. identified) | Type of Variant |
|------|------------------------------|---------------|------------------------------------------|-----------------|
| **APC** | 10 | 2 (20) | c.3352A>G (p.Asn1118Asp) | Missense |
| | | | c.6821C>T (p.Ala2274Val) | Missense |
| **ATM** | 105 | 32 (30) | c.1229T>C (p.Val410Ala) (7) | Missense |
| | | | c.4388T>G (p.Phe1463Cys) (6) | Missense |
| | | | c.6067G>A (p.Gly2023Arg) (5) | Missense |
| | | | c.1810C>T (p.Pro604Ser) (4) | Missense |
| | | | c.2932T>C (p.Ser978Pro) (2) | Missense |
| | | | c.4424A>G (p.Tyr1475Cys) (2) | Missense |
| | | | c.1066-6T> (IVS8-6 T>G) | Missense |
| | | | c.1073G>A (p.Asn358Ser) | Missense |
| | | | c.4709T>C (p.Val1570Ala) | Missense |
| | | | c.7390T>C (p.Cys2464Arg) | Missense |
| | | | c.8734A>G (p.Arg2912Gly) | Missense |
| **BARD1** | 23 | 8 (35) | c.222G>A (p.Ser761Asn) (4) | Missense |
| | | | c.33G>T (p.Gln11His) | Missense |
| | | | c.1694G>A (p.Arg565His) | Missense |
| | | | c.1977A>G (p.Arg659=) | Synonymous |
| | | | c.2191C>G (p.Arg731Gly) | Missense |
| **BMPR1A** | 2 | 2 (100) | c.1243G>A (p.Glu414Lys) | Missense |
| **BRCA2** | 23 | 3 (12) | c.6903G>A (p.Arg2298Lys) | Missense |
| | | | c.10121C>T (p.Thr3374lle) | Missense |
| | | | c.1514T>C (p.Ile505Thr) | Missense |
| **BRIP1** | 33 | 1 (3) | c.587A>G (p.Asn196Ser) | Missense |
| **CDH1** | 19 | 2 (10) | c.670C>T (p.Arg224Cys) | Missense |
| | | | c.1747G>A (p.Ala582Thr) | Missense |
| **CDKN2A** | 5 | 1 (20) | c.361G>A (p.Ala121Thr) | Missense |
| **CHEK2** | 117 | 5 (4) | c.320-5T>A (2) | Intron variant |
| | | | c.538C>T (p.Arg180Cys) (2) | Missense |
| | | | c.707T>C (p.Leu236Pro) | Missense |
| | | | c.345+6A>T | Missense |
| | | | c.632C>G (p.Pro211Arg) | Missense |
| | | | c.178G>A (p.Val60Ile) | Missense |
| **FANCC** | 3 | 3 (100) | c.2252A>G (p.Lys751Arg) | Missense |
| | | | c.1475C>A (p.Ala492Asp) (2) | Missense |
| **MLH1** | 7 | 1 (17) | c.2252A>G (p.Lys751Arg) | Missense |
| **MRE11A** | 8 | 2 (25) | c.1475C>A (p.Ala492Asp) (2) | Missense |
| **MUTYH** | 22 | 1 (4) | c.1276C>T (p.Arg426Cys) | Missense |
| **NBN** | 20 | 2 (10) | c.1720T>A (p.Leu574Ile) (2) | Missense |
| **NF1** | 4 | 1 (25) | c.8041A>G (p.Ile2681Val) | Missense |
| **PALB2** | 49 | 6 (12) | c.2228A>G (p.Tyr743Cys) | Missense |
| | | | c.2816T>G (p.Leu939Trp) (3) | Missense |
| | | | c.656A>G (p.Asp219Gly) (2) | Missense |
| | | | c.986>G>C (p.Gly329Ala) (2) | Missense |
| | | | c.620G>A (p.Gly207Cys) | Missense |

(continued on following page)
relevant clinical interpretations of findings, as well as the evidence on which interpretations are based. To better identify the underlying reasons for their discrepant interpretation, laboratories could explicitly describe their weighting (from very strong to moderate or supporting) for each pathogenic criterion used for classification. This transparency in data sharing and collaboration between academic research consortia and commercial laboratories may promote strategies to standardize clinical variant curation algorithms. Ongoing consortia such as Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), and International Society for Gastrointestinal Hereditary Tumors (InSIGHT) have demonstrated the utility of multidisciplinary collaboration to curate and reclassify submitted VUS. Platforms such as the Leiden Open Source Variation Database (LOVD), which allows for collection, curation, and display of phenotypes and DNA sequence variants, and the BRCA Challenge (http://brcaexchange.org/), an international effort to review and provide vetted data on BRCA1 and BRCA2 gene variants) are additional relevant resources. PROMPT uses

| Gene | No. With Multiple Submissions | Total No. (%) | Description of Variants (No. identified) | Type of Variant |
|------|-------------------------------|---------------|------------------------------------------|----------------|
| RAD50 | 17                           | 4 (24)        | c.3036+5G>A (3) c.943G>T (p.Val315Leu)   | Missense       |
| RAD51C | 26                          | 12 (46)       | c.790G>A (p.Gly264Ser) (12)               | Missense       |
| STK11 | 2                            | 1             | c.1211C>T (p.Ser404Phe)                   | Missense       |
| TP53  | 15                           | 5 (33)        | c.248C>T (p.Ala83Val) c.704A>G (p.Asn235Ser) | Missense       |
| TSC2  | 2                            | 1             | c.5378G>A (p.Arg1793Gln)                  | Missense       |

| Gene | No. With Multiple Submissions | Total No. (%) | Description of Variants (No. identified) | Type of Variant |
|------|-------------------------------|---------------|------------------------------------------|----------------|
| CHEK2 p.S428F | x | x | x | x* |
| Functional studies, from literature review, supportive of a damaging effect (very strong evidence) | x | x | x | x† |
| Increased prevalence in affected individuals vs control subjects (strong evidence) | x | x | x | x |
| Location in a well-established functional domain (moderate evidence) | x | x | x | x |
| Cosegregation with disease in multiple affected family members (supporting evidence) | N/A | x | x | N/A |
| Prediction by in silico algorithms (supporting evidence) | x | N/A | N/A | N/A |
| Contribution to tumorigenesis determined by LOH | N/A | x | N/A | N/A |
| Final interpretation | Pathogenic | Pathogenic (low penetrance) | Pathogenic | Uncertain clinical significance |

| Gene | No. With Multiple Submissions | Total No. (%) | Description of Variants (No. identified) | Type of Variant |
|------|-------------------------------|---------------|------------------------------------------|----------------|
| CHEK2 p.I157K | x | x | x | x‡ |
| Functional studies, from literature review, supportive of a damaging effect (very strong evidence) | x | x | x | x‡ |
| Increased prevalence in affected individuals vs control subjects (strong evidence) | x | x | x | x§ |
| Location in a well-established functional domain (moderate evidence) | x | x | x | x |
| Cosegregation with disease in multiple affected family members (supporting evidence) | x | N/A | x | N/A |
| Prediction by in silico algorithms (supporting evidence) | Inconsistent | N/A | Limited value | N/A |
| Contribution to tumorigenesis determined by LOH | N/A | N/A | N/A | N/A |
| Final interpretation | Expected pathogenic | Pathogenic (low penetrance) | Pathogenic | Uncertain clinical significance |

Abbreviations: LOH, loss of heterozygosity; N/A, not applicable.
*Interpretation of variable impairment.
†Interpretation of increased risk only in specific populations.
‡No effect on CHEK2 protein kinase activity, and relationship between experimental findings and cancer association is unclear.
§Observed at high frequencies among controls in some particular populations.
¶Incomplete segregation.
both crowd-sourcing and direct participant enrollment; therefore, it also can provide a platform for cohort formation and prospective follow-up of individuals and their family members harboring genetic variants. However, at this time, health-care providers and patients need to be aware that there could be conflicting interpretations of variants and those variants may be reclassified.

Our analysis has limitations. Because PROMPT is an elective, patient-oriented registry, there may be significant ascertainment bias. For example, individuals with VUS or those who were self-aware of a variant with conflicting interpretation may have been more likely to report their variant to PROMPT, leading to an overestimation of discrepant findings. On the other hand, a significant number of test results reported by participants were not submitted to ClinVar by any laboratory and, therefore, were excluded from the primary analysis, potentially leading to a mis-estimate of conflicting variant interpretations. Finally, because some patients did not upload their clinical test report, it was not possible to carry out more in-depth analysis in many cases. Efforts to obtain reports from all participants are ongoing.

In conclusion, clinical interpretation of genetic testing for increased cancer susceptibility as assessed by multiplex panels hinges on accurate curation and interpretation of variants. Discrepant interpretation of some genetic variants appears to be common. Internet-based registries provide a powerful tool to collect data to inform efforts to standardize classification of genetic variants, and can play an important role in efforts to minimize potential medical harms due to false alarm or false reassurance following cancer genetic testing.

DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

**Fig A1.** Type of conflicting interpretation by genes.