Genetic Testing is Not “One and Done”

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In 1996, one commercial laboratory began germline testing for *BRCA1* and *BRCA2*,¹ two highly penetrant genes. Fast forward to today, when multiple laboratories offer testing panels that include dozens of genes.² Many of these genes have only low or moderate penetrance, so different pathogenic variants in the same gene may cause more cancer risk—and therefore be more clinically important—than others. In addition, data demonstrating genotype–phenotype correlation for some genes are lacking. Finally, variants in any gene may be reported as being of uncertain significance. This expanded testing therefore requires more nuanced interpretation³ and the need for variant classification tools.

ClinGen is a collaboration of experts, professional societies, and commercial laboratories. It uses standardized terminology (benign, likely benign, of uncertain significance, likely pathogenic, and pathogenic)⁴ to classify gene variants submitted to ClinVar, its archive of genomic data.⁵ ClinVar makes variant information publicly available to improve our understanding of genotype–phenotype correlation.^{5,6} Even with robust data sharing, however, many variants have conflicting interpretations of pathogenicity.

Yin et al.⁷ report their review of variant pathogenicity concordance between laboratory and ClinVar interpretations. The 7496 variants included in their study were sequenced between 1996 and 2019 and reported to 11 clinical practices. Duplicates and variants that did not meet study inclusion criteria were excluded, leaving 4798 unique variants for analysis, 77% of which had entries in ClinVar

(and were therefore considered mappable to ClinVar). Not all laboratories report their findings to ClinVar, explaining the remainder, unmappable to ClinVar.

Half of the unmappable variants were considered pathogenic by the reporting laboratory, versus 20.8% of variants mapped to ClinVar. Unmappable variants were significantly less likely to be reported as variants of uncertain significance (VUSs) (44.8% versus 74.0%) or to be inconsistently interpreted (0.8% versus 3.4%), which makes sense because the unmappable variants were interpreted by a single laboratory.

Of the 770 mappable variants reported by any laboratory as pathogenic, 718 were also considered pathogenic in ClinVar. Of the rest, 1 was considered benign, 22 were considered VUSs, and 29 had conflicting interpretations in ClinVar. Ten of the 2738 variants reported by laboratories as VUSs were reported in ClinVar as pathogenic, and 475 had conflicting interpretations. Two of 66 laboratory-reported benign variants were considered pathogenic in ClinVar. Altogether, 21.3% of mappable variants had ClinVar interpretations discordant with those reported by laboratories.

Yin et al. are not the only authors to find discordance. Gradishar et al.⁸ interrogated ClinVar and identified 4250 *BRCA1* and *BRCA2* variants, for which pathogenicity matched that determined by a reference commercial laboratory in only 73.2% of cases, while 12.3% were partially concordant. Variants reported in ClinVar as VUS, benign, or pathogenic had different reference laboratory classifications in 14.5% of cases.⁸

Balmana et al.⁹ evaluated 603 variants reported by 518 individuals enrolled in the Prospective Registry of Multiplex Testing (PROMPT); 165 of these people provided results from a laboratory that does not submit data to ClinVar. Of the 603 variants, 26% were reported in ClinVar as having conflicting interpretations; *CHEK2* and *ATM* accounted for almost half of those. Of 155 variants with

conflicting interpretations, 56 (36%) were clinically significant (determined to be pathogenic/likely pathogenic by at least one but not all laboratories).⁹

This discordance may cause lifelong consequences for patients. In *The Wall Street Journal* in December 2019, Amy Dockser Marcus reported the story of a family identified as having a pathogenic *BRCA2* variant. Members of this family later learned that the variant was reclassified—downgraded by the original lab—after many of them had undergone prophylactic surgeries.¹⁰ Interestingly, and reflective of the discordance noted by Yin et al.,⁷ other laboratories continue to report the family *BRCA2* variant as likely pathogenic.¹¹ Regardless, this was unnerving for patients and providers who use genetic test results to make (potentially irreversible) decisions.

There are a few reasons for this disagreement. While variant classification guidelines exist,⁴ different laboratories have their own testing methods, multiple variant classification tools exist, and results require interpretation, which may be subjective.^{12,13} In addition, variant data are lacking for certain ethnicities, limiting classification. Some data are considered proprietary and therefore not shared with the wider research community. In response, professional societies, including the American College of Medical Genetics and Genomics, have strongly encouraged data sharing and collaboration in variant classification.^{11,14,15}

How, then, do we take care of the patient in front of us in the setting of variant interpretation uncertainty? Going back to smaller gene panels is unlikely to solve the problem. VUSs continue to be identified even in *BRCA1* and *BRCA2*^{7,8} and are subject to reinterpretation. From 2006 to 2016, for example, one commercial laboratory performed 1.67 million hereditary cancer tests, of which 5.8% reported at least one VUS; *BRCA2* and *ATM* were most frequently reported as VUSs and then reclassified.¹⁶

We frequently order genetic testing, and because we use these results to manage patients,^{17,18} we should know the limitations of testing and should pursue continuing medical education activities offered by professional societies, including the Society of Surgical Oncology and the American Society of Breast Surgeons. It is important to ask laboratories about their variant classification methods, and how they reclassify variants and notify patients or providers of changes. We can utilize tools such as VarHarmonizer,^{7,19} described by Yin et al. to keep up with changing variant classifications, and we should develop systems within our own practices for notifying patients or their next of kin of any changes.^{20,21}

Genetic test interpretation is no more black-or-white than imaging interpretation is for a radiologist. I propose that we approach germline test as we do most other tests we order, taking the results into context based on the patient in

front of us and with the knowledge that technology improves over time. The landscape is rapidly changing, and clinically significant reclassification is always a possibility. As we counsel patients, we should be wary of speaking in absolutes. Finally, to improve the care of our patients, we need to encourage data sharing by commercial and research laboratories, which will advance efforts to better classify and understand these germline variants.

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