original reports abstract

Germline Testing Data Validate Inferences of Mutational Status for Variants Detected From Tumor-Only Sequencing

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PURPOSE Pathogenic germline variants (PGVs) in cancer susceptibility genes are usually identified through germline testing of DNA from blood or saliva: their detection can affect treatment options and potential risk-reduction strategies for patient relatives. PGV can also be identified in tumor sequencing assays, which, when performed without patient-matched normal specimens, render determination of variants' germline or somatic origin critical.

METHODS Tumor-only sequencing data from 1,608 patients were retrospectively analyzed to infer germline versus somatic status of variants using an information-theoretic, gene-independent approach. Loss of heterozygosity was also determined. Predicted mutational models were compared with clinical germline testing results. Statistical measures were computed to evaluate performance.

RESULTS Tumor-only sequencing detected 3,988 variants across 70 cancer susceptibility genes for which germline testing data were available. We imputed germline versus somatic status for > 75% of all detected variants, with a sensitivity of 65%, specificity of 88%, and overall accuracy of 86% for pathogenic variants. False omission rate was 3%, signifying minimal error in misclassifying true PGV. A higher portion of PGV in known hereditary tumor suppressors were found to be retained with loss of heterozygosity in the tumor specimens (72%) compared with variants of uncertain significance (58%).

CONCLUSION Analyzing tumor-only data in the context of specimens' tumor cell content allows precise, systematic exclusion of somatic variants and suggests a balance between type 1 and 2 errors for identification of patients with candidate PGV for standard germline testing. Although technical or systematic errors in measuring variant allele frequency could result in incorrect inference, misestimation of specimen purity could result in inferring somatic variants as germline in somatically mutated tumor suppressor genes. A user-friendly bioinformatics application facilitates objective analysis of tumor-only data in clinical settings.

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INTRODUCTION

Precision oncology relies on robust molecular analyses of patient samples and accurate interpretation of genomic sequencing and biomarker data.[1] Advances in genomic sequencing have made tumor genomic profiling a routine process in the clinical evaluation and treatment planning of patients with cancer.[2] The main objective is to provide a detailed characterization of a patient's neoplasm, improve predictions on clinical outcome, and identify and potentially target oncogenic drivers to enable the development of an individualized treatment plan.[3]

A small but important set of cancers arise in patients with pathogenic germline variants (PGVs) that can both inform personal and familial cancer risks and guide treatment approaches.[4] Clinical germline testing has typically been limited to patients with personal and/or family history of tumors highly suggestive of specific predisposition syndromes. However, germline testing is now expanding to a larger group of patients beyond those with a compelling family history.[5] In addition, for a patient to be referred for clinical germline testing, certain features are often required by health insurance companies, which can restrict uptake. Because of complexities in determining the need for clinical germline testing, eligible patients are frequently overlooked and not tested.[6] A recent study showed that one in eight adult patients with cancer who underwent universal germline testing, regardless of the extent to which they met established criteria, had a PGV in a susceptibility gene.[7] Almost half of these PGVs would not have been identified if testing criteria had been

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

The increase in availability and demonstrated efficacy of tumor sequencing assays has led to their routine use for precision oncology. However, patient-matched DNA is often lacking in clinical settings. When a potentially pathogenic mutation in a tumor suppressor gene is detected, divergent but important clinical implications require that its germline or somatic origin be determined and evidence for the loss of the wild-type allele be resolved.

Knowledge Generated

An information-theoretic, gene-independent approach accurately inferred mutational status of pathogenic variants in tumor-only data using sequencing information commonly available in clinical reports. Analyzing detected variant in the context of specimen purity, eliminated the need for ad hoc criteria, and allowed predicting loss of heterozygosity.

Relevance

Pathogenic germline variants are detected in diverse malignancies including those not traditionally associated with cancer predisposition syndromes. Imputing mutational status and distinguishing biallelic loss from monoallelic alterations provides additional insight for interpreting and therapeutically targeting variants detected by tumor-only sequencing.

followed. Furthermore, approximately one third of the PGV carriers had their therapies changed as a result.

Tumor sequencing for identification of somatic alterations is becoming more widely carried out in patients with different cancers.[8,9] Many commercial and academic tumor sequencing assays include a large set of cancer-related genes (> 50) that can be mutated somatically and also confer cancer risk when mutated in the germline. To definitively identify somatic variants and potential germline variants in cancer cells, some laboratories analyze matched tumor and nontumor specimens (eg, blood, buccal mucosa, and adjacent tissue).[8] Multiple studies have shown that integration of tumor sequencing and matched normal genomic profiling can identify PGV in cancer predisposition genes in 15%-18% of patients with cancer, including those without high-risk family history or otherwise meeting clinical criteria for standard germline testing.[10,11] These data suggest that current germline testing strategies may miss a significant number of mutation carriers in the population that are not identified by the patients' and/or family history.

Although concomitant tumor and germline sequencing analyses for all patients with cancer may eventually become the standard of care in the future, an objective and reliable means of identifying patients for clinical germline testing confirmation is needed in the clinic today.[12] Current practices in interpreting tumor-only data for this purpose are gene-specific and are often on the basis of variant allele frequency (VAF) criteria that may need to be adjusted for different settings and tumor types.[13,14] Although such criteria could be sensitive for identifying PGV from tumor-only data,[14-16] their application requires extensive expert review and results in low specificity in exclusion of somatic variants and a significantly decreased overall accuracy.[12,13] To address these challenges, we examined the performance of a gene-independent, information-theoretic pipeline aimed at accurately categorizing the variants identified by tumor-only assays as somatic or germline. Using commonly available sequencing data, we analyzed each variant in the context of specimen's proportion of tumor cells and used high-depth sequencing to predict loss of heterozygosity (LOH) status, which can potentially inform the functional effect of the mutation in both germline and somatic variants.

METHODS

The cohort included a total of 1,608 patients with diverse malignancies who were consented to the PROFILE study[17] (institutional review board–approved protocols 11-104 and 17-000) at Dana-Farber Cancer Institute between January 2014 to December 2018 and had undergone somatic sequencing. These patients had also received clinical germline testing, the results of which were collected, queried, and deidentified (institutional review board–approved protocols 19-354 at Dana-Farber Cancer Institute and 2019002191 at Rutgers). Nomenclature variations between tumor sequencing and germline testing data were curated for 70 overlapping genes between the assays by comparing the reference transcript number, the position, and the type of the alteration in the specific genes. Pathogenicity of germline variants was assessed using curated open-access US Food and Drug Administration–approved knowledge bases (ClinVar/ClinGen) and the American College of Medical Genetic guidelines.[18] Pathogenic status and mutational effect of somatic variants were assessed using the oncoKB annotator.[19] The proportion of tumor cells (purity) and its confidence intervals were computationally estimated for all specimens using All-FIT[20] using VAFs, sequencing depth, and copy number of all single-nucleotide variants (SNVs) and indels, which are commonly available in clinical tumor sequencing reports.

Computational and histologic purity estimates were used to infer germline versus somatic status and evaluate LOH for SNV and indels using LOHGIC[21] taking into account the uncertainties in estimates of purity and VAF, which depend on sequencing depth (Data Supplement). Genomics

Oncology Platform is a python GUI, designed on the basis of the workflow implemented in this study (Data Supplement), and is freely available,[22] for the extraction of relevant information and the application of All-FIT and LOHGIC directly on variant calls.

RESULTS

There were a total of 1,467 eligible patients. They had both tumor sequencing and independent germline sequencing results (Table 1, Fig 1), were predominantly female (73%), were White or Caucasian non-Ashkenazi (84%), and had a median age of 54 years (range, 1-88 years) at first primary tumor diagnosis. The most frequent tumor types were breast (22%), epithelial ovary including fallopian tube and peritoneum (21%), and colorectal cancers (19%). A total of 725 patients (49%) had reportable germline findings, 285 (29%) of whom had at least one PGV and 440 (61%) had exclusively one or more variants of uncertain significance (VUS). Individuals self-identified as Ashkenazi Jewish had a high rate of PGV (38 of 44; 13% of all PGV) in contrast with Hispanic individuals who had the lowest rate (0.7%) in our cohort. The approximate frequency of PGV in genes associated with the sequenced tumor were small bowel carcinoma, 29%; urothelial carcinoma, 25%; renal cell carcinoma, 24%; colorectal carcinoma, 15%; breast carcinoma, 14%; epithelial ovarian carcinoma, 13%; and pancreatic adenocarcinoma, 13%. No PGVs were detected in 35 genes analyzed; 1-3 PGV were detected in 22 genes, and >3 PGV were detected in 13 genes (Data Supplement).

Tumor-only sequencing using the OncoPanel assay detected 5,426 variants across 70 cancer susceptibility genes[23,24]; matched germline testing results of the relevant gene was available for 3,988 of them. In total, 728 variants were detected by germline testing among which 231 were annotated as PGV and 497 as VUS. The remaining 3,260 variants were not reported in germline analysis and therefore were deemed to be somatic (Fig 2A, Data Supplement); 1,792 of these variants (55%) were predicted to be likely pathogenic or pathogenic.

We inferred nonambiguous, germline, or somatic mutational status for 3,028 (75.9%) variants using computational estimates of specimen tumor purity and 3,173 (79.5%) variants using histologic estimates (Fig 2B, Data Supplement). Computational estimates were significantly correlated with specimen histologic assessments of tumor purity (Pearson $r=0.31$; $P<.001$). As such, inferred mutational status using either purity estimate were highly concordant (Jaccard index = 0.84[25]). The performance results using computational and histologic purity estimates were also highly concordant (Fig 3, Data Supplement). For simplicity, the remainder of the results will only report those from computational estimates, which are calculated as a part of our pipeline.

We evaluated the accuracy of inference results considering all germline variants (PGV and VUS) or only the PGV, along with somatic variants. When only the PGV and pathogenic somatic variants were considered, sensitivity (true positive rate) was 65%, signifying the rate at which the PGV were correctly inferred. Specificity (true negative rate), which indicates the rate of correctly inferring true somatic variants, was 88%. The false omission rate, indicating how often a true PGV was incorrectly inferred as somatic was

TABLE 1. Patient Demographic and Clinical Characteristics (N = 1,467)

Characteristic	No.	%
Sex		
Female	1,075	73
Male	392	27
Self-reported race or ethnicity		
White or Caucasian (non-Ashkenazi)	1,237	84
Ashkenazi Jewish	44	3
Black or African American	40	3
Asian	48	3
Hispanic	28	2
Other or mixed	43	3
Unknown	25	2
Cancer diagnoses		
Single primary	1,122	76
Multiple primaries	345	24
Age at first cancer diagnosis, years		
0-19	24	2
20-29	66	4
30-39	166	11
40-49	314	21
50-59	429	29
60-69	301	21
70-79	131	9
≥ 80	20	1
Unknown	16	1
Age at germline test, years		
0-19	11	1
20-29	43	3
30-39	125	9
40-49	241	16
50-59	400	27
60-69	400	27
70-79	205	14
≥ 80	42	3
No. of genes tested in the germline		
Single-site testing (1 or more sites)	14	1
1-5	64	4
6-15	33	2
16-50	1,001	68
≥ 51	355	24

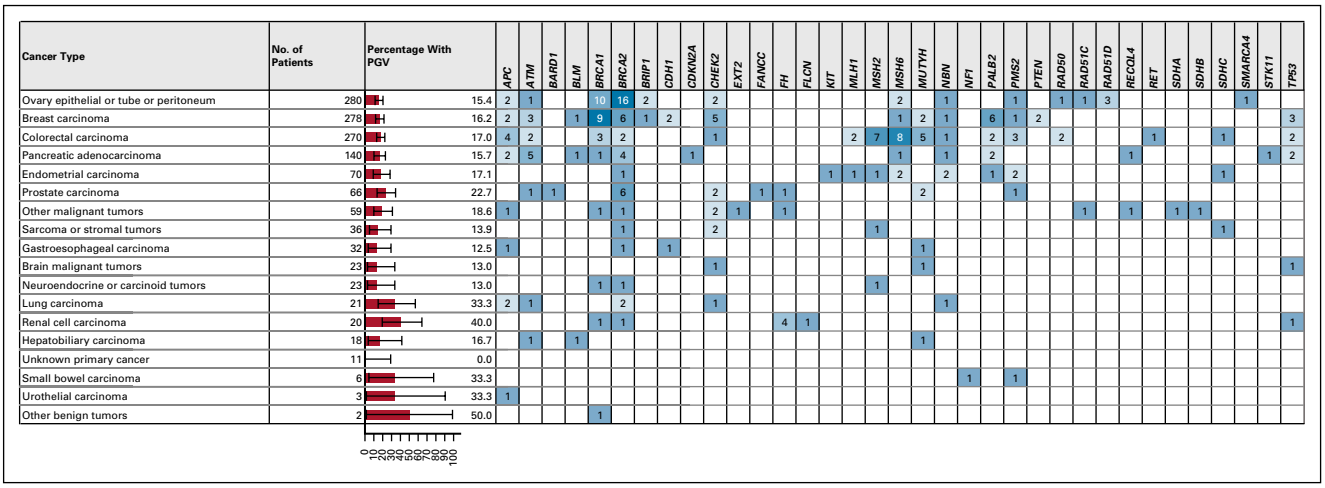

FIG 1. The percentage of tumors per cancer type with PGVs in 35 genes from germline testing. PGV, pathogenic germline variant.

only 3%. Precision (positive predictive value) and the F-score were 31% and 42%, respectively, which could be attributed to the relatively low number of true PGV in the data set compared with the number of true somatic variants. The overall accuracy of the analysis was 86%. These results did not change when pathogenicity of variants was considered (Data Supplement).

The majority of somatic variants that were incorrectly inferred as germline (278 of 394, 71%) had VAF > 50% (Fig 4A), whereas 83% of true PGV (118 of 143) that were incorrectly inferred to be somatic had VAF < 50% (Fig 4B). In the latter group, 22% of incorrectly inferred variants corresponded to indels. There was no significant difference between the focal copy number or the types of variants—SNV or indel—with correct or incorrect inference. The percentage of variants with ambiguous inference was 20.5% and 24.1% using computational and histologic purity estimates, respectively. Variants with ambiguous

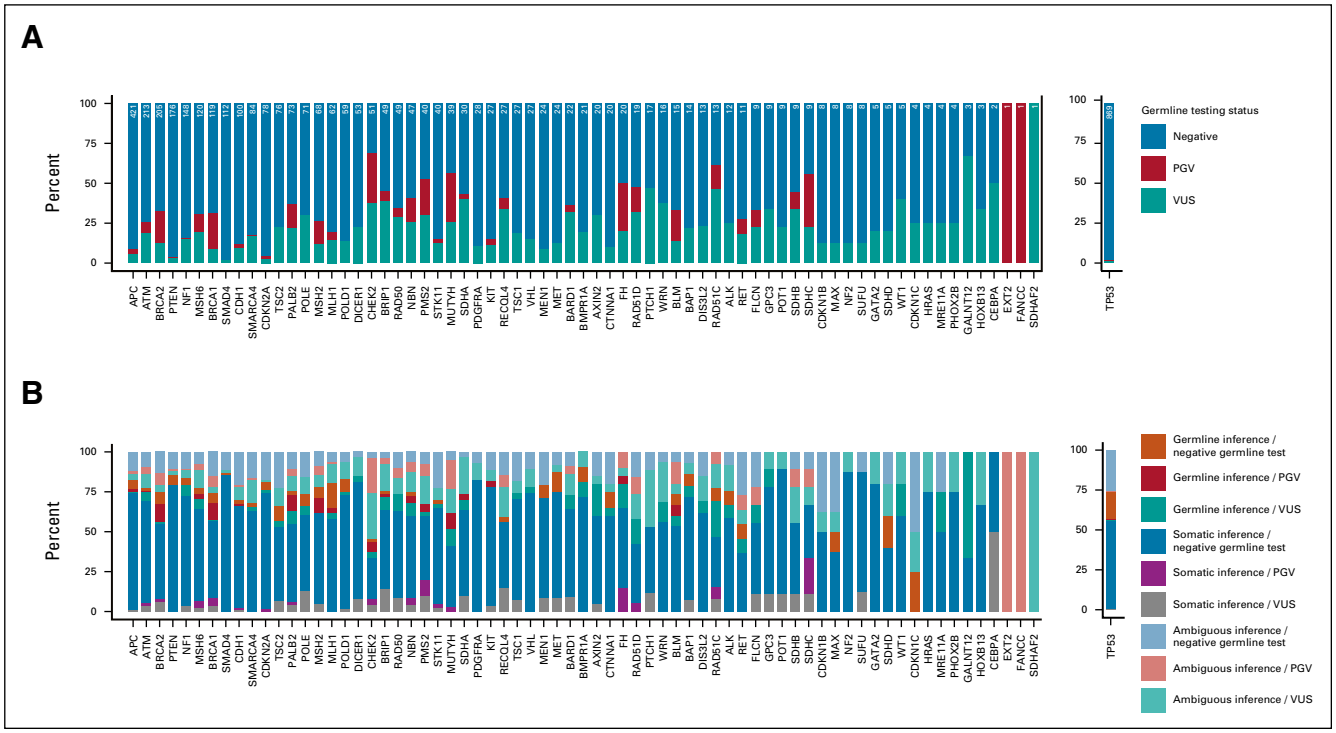

FIG 2. (A) Matched germline testing results for 3,988 variants detected by tumor-only sequencing in 70 cancer susceptibility genes, including 231 PGV, 497 germline VUS, and 3,260 somatic variants. (B) Inference of mutational status using computational purity estimates compared with germline testing results. The results using histologic purity estimates are shown in the Data Supplement. PGV, pathogenic germline variant; VUS, variants of uncertain significance.

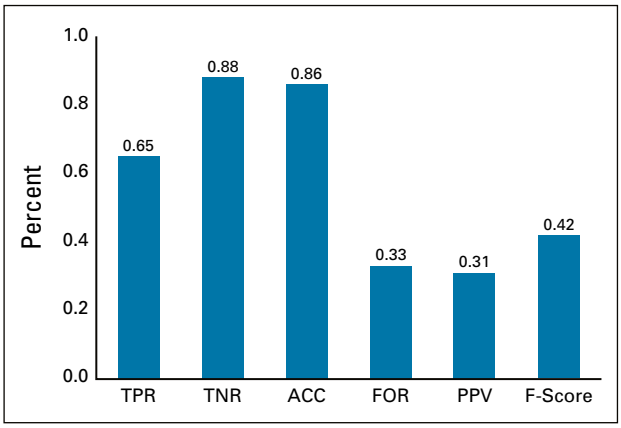

FIG 3. TPR (or recall), TNR, ACC, FOR, PPV (or precision), and F-score performance measures for the inferences made using computational purity for PGVs versus pathogenic somatic variants. The result did not change when pathogenicity of variants was considered (Data Supplement) or using histologic purity estimates (Data Supplement). ACC, accuracy; FOR, false omission rate; PGV, pathogenic germline variant; PPV, positive predictive value; TNR, true negative rate; TPR, true positive rate.

inference had a mean VAF of 52.2% (Fig 4C, Data Supplement). Expected allele frequency for germline heterozygous mutations is 50% and is independent of tumor purity; however, various somatic models also predict expected VAF of 50% across a range of purity and copy-number values (Data Supplement), which could result in ambiguous inference.

Mean sequencing depth of variants with correct predictions (mean = 295.5; standard deviation [SD] = 147.1) was not significantly different from that of variants with incorrect inferences (mean = 283.7; SD = 149.6). Similarly, purity estimates were not significantly different in specimen with variants that were inferred correctly (mean = 48.1; SD = 21.4) versus incorrectly (mean = 44.8; SD = 23.9). Although low sequencing depth and inaccurate purity estimation can contribute to the false inference of variants, they did not systematically bias the performance.

Somatic mutations in *TP53* are the most common alterations in human cancers, whereas germline *TP53* mutations, the underlying cause of Li-Fraumeni syndrome, are rare. We correctly inferred mutational status of germline mutations in five of five Li-Fraumeni syndrome cases. Peripheral blood sequencing for germline testing was also positive for three additional cases; however, the VAFs of these variants in blood and tumor were 6%-18%, suggesting detection of mosaicism because of clonal hematopoiesis.[26] Moreover, 17.6% (150 of 852) of *TP53* variants detected by tumor sequencing were falsely inferred to be germline. These variants were detected at VAFs significantly higher than their respective specimens' estimated purity (rank-sum test $P < .001$, Data Supplement). Similarly, significant patterns were also observed for incorrectly inferred somatic variants in *APC* (rank-sum test $P = .018$) and *PTEN* (rank-sum test $P = .003$), implying that inference of variants with high VAF in tumor suppressor genes may be affected by inaccuracies in estimating purity and confounded by unreported focal copy-number changes from loss of the wild-type allele or copy-neutral LOH.

Before inferring mutational status, the overall proportion of the PGV to all pathogenic variants detected by tumor-only sequencing was 11% (1%-100% for individual genes, Data Supplement). When only the pathogenic variants with VAF > 30% were considered,[14] this ratio increased to 19%, resulting in a sensitivity of 91% for detection of true germline variants, a specificity of 50% for detection of pathogenic somatic variants, and an overall accuracy of 55%. By contrast, our model's nonambiguous, correct inference for 71% of pathogenic somatic variants increased the ratio of PGV to remaining pathogenic variants to 31%, without imposing any VAF criteria.

Next, we assessed the likelihood for the loss of the wild-type allele or copy-neutral LOH for all germline and somatic variants with correctly inferred mutational status. In total, a significantly larger percentage of PGV (72%) had LOH compared with 58% of germline VUS (chi-squared $P <$ .001) and 39% of pathogenic or likely pathogenic somatic variants (chi-squared $P < .001$) (Fig 5A). The prevalence of PGV with LOH was evident when we focused on the genes associated with specific cancers, including those with high and moderate or low penetrance.

The high-penetrance genes associated with hereditary breast cancer include *BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *STK11*, and *TP53*, whereas *ATM* and *CHEK2* are considered as moderate or low penetrance.[27] In females, LOH was demonstrated for *BRCA1* PGV and *BRCA2* PGV in 86% (6 of 7) of breast and 94% (15 of 16) of ovarian tumors, whereas LOH was demonstrated for only 33% of *BRCA1/2* PGV (2 of 6) in other tumor types (Fig 5B). LOH was demonstrated for all PGV (13 of 13) in *PALB2*, *TP53*, *ATM*, *CDH1*, and *CHEK2* in all tumor types. In males, LOH was demonstrated for *BRCA2* PGV in 83% (5 of 6) of pancreatic and prostate tumors. These results agree with known prevalence of PGV in these genes for corresponding cancer types.[28-30]

The high-penetrance *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes are associated with Lynch syndrome.[31] LOH was evident for 78% of PGV (7 of 9) in colorectal cancers of both sexes. In females, LOH was demonstrated for *MSH6* PGV in ovarian tumors (2 of 2; Fig 5C). Considering both sexes, 79% of PGV in the Lynch syndrome genes were found with LOH (11 of 14) across all tumor types. The results are again consistent with the status of pathogenic alterations associated with the Lynch syndrome particularly for ovarian cancer in females and colon cancer in males. By contrast, somatic variants in the genes associated with the hereditary breast cancer or Lynch syndrome did not show a significant correlation between inferred LOH and pathogenicity, although other events resulting in biallelic inactivation could not be ruled out.

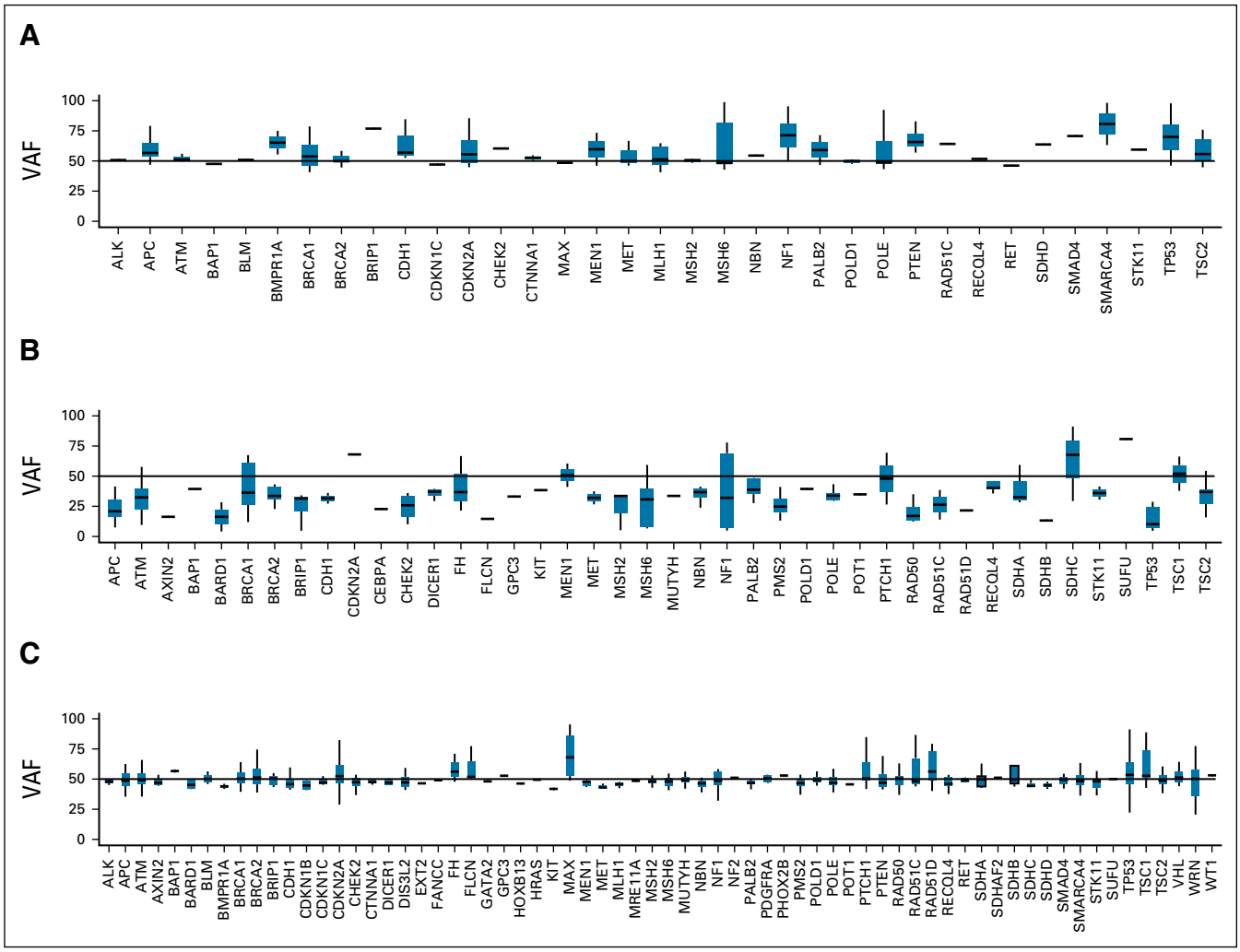

FIG 4. Allele frequency distribution of variants with incorrect or ambiguous inference per gene: (A) somatic variants with germline inference, (B) germline variants with somatic inference, and (C) germline and somatic variants without a statistical inference (ambiguous). The results using computational purity estimates are shown; the results from histologic purity estimates are shown in the Data Supplement. PGV, pathogenic germline variant; VAF, variant allele frequency; VUS, variants of uncertain significance.

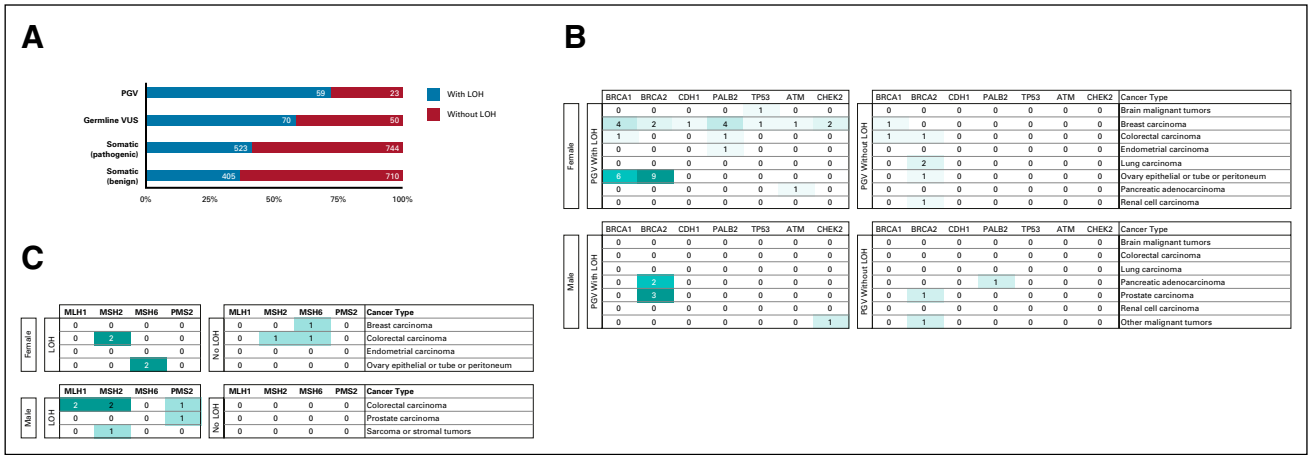

FIG 5. (A) Percentage of PGV, germline VUS, and somatic pathogenic and benign variants with and without LOH in all genes. (B) Prevalence of PGV with and without LOH per cancer type for genes associated with breast cancer in females and males. (C) Prevalence of PGV with and without LOH per cancer type for genes associated with Lynch syndrome in females and males. LOH, loss of heterozygosity; PGV, pathogenic germline variant; VUS, variants of uncertain significance.

DISCUSSION

Estimates of the prevalence of inherited susceptibility to cancer are still imprecise in the general population. Emerging data from clinical sequencing assays indicate that the incidence of PGV may be as high as 17.5% in unselected patients with cancer, and even higher for specific histologic types,[32,33] reflecting the dependence on penetrance and tissue specificity.[34]

Extracting clinically relevant information from sequencing data requires accurate annotation of somatic and germline alterations by comparing a tumor's molecular profile with the patient-matched normal samples, which can also help identify somatic events that affect the genes with a PGV resulting in biallelic inactivation.[35] However, most commercial and academic laboratories lack control germline DNA analysis and produce reports that may not address whether or not a variant is actually somatic. Here, we presented a gene-independent bioinformatics workflow that using commonly available measurements from tumor sequencing (ie, total depth, focal ploidy, and VAF) can select the most likely germline versus somatic mutational status and assess evidence for LOH (Data Supplement). By analyzing each variant in the context of specimen purity, we eliminate the need for ad hoc VAF criteria,[14] or complex analyses of raw sequencing data.[36]

In a clinical sense, the cost of misclassifying a true somatic mutation (type 1 error) is equal to the cost of performing germline testing, which can correct the incorrect inference results. However, the cost of failing to identify the presence of a germline mutation (type 2 error) may result in neglecting to validate the mutational status through germline testing and possibly leaving the treating physician without critical information that could alter the treatment strategy and missing the opportunity for cascade testing of at-risk family members. Gene-specific, VAF-based criteria for identifying patients with PGV from tumor-only data prioritize sensitivity at the cost of specificity; their application results in a high number of type 1 errors, and thus, low overall accuracy.[12] In our data, only 11% of all detected pathogenic variants were PGV. By contrast, accurate inference of status for 71% of true pathogenic somatic variants led to a three-fold increase in the proportion of PGV to remaining pathogenic variants and established a balance between sensitivity and specificity for distinguishing germline variants from somatic variants.

Following the Knudson two-hit model, tumorigenesis in PGV carriers is caused by the presence of a heterozygous germline alteration followed by the somatic loss of the wild-type allele in the tumor cells, or more rarely epigenetic silencing.[37] As not all cancers that arise in carriers may be driven by the germline alteration, it is important to determine whether a germline variant is accompanied by LOH in a given cancer, both to understand the pathogenesis and to guide therapy. Our results showed a significant association between pathogenicity of germline alterations and LOH, highlighting the importance of distinguishing biallelic events from monoallelic PGV as a biomarker for therapeutic response.[38] In particular, the high rates of inferred LOH for pathogenic *BRCA1* and *BRCA2* variants in breast and ovarian cancers in our data are consistent with similar findings using other sequencing platforms suggesting existence of selective pressures for biallelic inactivation in these tumors.[30,35]

Systematically, the lower the sequencing depths at which a particular variant is detected, the lower the confidence in accuracy of measuring its VAF. Clinical tumor-only sequencing assays are mandated to have a relatively high depth of sequencing compared with research-grade whole-genome and whole-exome platforms; therefore, they are capable of identifying SNV and indels with high confidence. Sequencing at depth of coverage $> 300\times$ is expected to provide sufficient power to accurately measure VAF and to statistically assess potential germline origin and zygosity of detected variants.[20,39] With an average coverage depth of approximately $290\times$ in our data, we did not observe a systematic difference in sequencing depth or specimen purity between the variants with true or false inferences. Our false omission rate of 3%, the result of lower-than-expected VAFs of PGV in tumor-only data, suggests either a problem in variant calling, undetected low-level amplification of the wild-type allele, or possibly presence of reversion mutations,[21,40,41] and underscores the dependency of our approach on accurate VAF measurements. The high VAF of confirmed somatic variants that were inferred as germline suggests a misestimation of purity, computationally and/or histologically. Although VAF for indels may be confounded by misalignment and variant calling inaccuracy, they were not associated with inference outcome, highlighting the utility of our approach for all variants with a measured VAF.

Independent validation of this information-theoretic methodology in larger, more diverse cohorts is required to not only further evaluate its performance, but also assess its usability and efficacy for medical decision making. Although cyclical hypothesis generation and evidence evaluation through germline testing and additional sequencing in the clinic can continually refine and validate the rigor of the pipeline, its inference results can be used in multigene prediction models, which, through the integration with clinical, familial, and ancestry information, may improve the probability of PGV identification.

In conclusion, our analysis demonstrates that patients with potential PGV in cancer predisposition genes can be identified by analyzing their tumor-only sequencing data; therefore, when a PGV is detected in a tumor specimen, the patient should be considered for genetic counseling and germline testing. Our results also demonstrate the utility of our approach for personalized clinical practice, even in the setting of paired tumor-normal sequencing, by evaluating allelic status and LOH for pathogenic germline and somatic variants as a critical therapeutic biomarker.

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N.J. and I.G. contributed equally to this work. J.E.G. and H.K. were coleaders of this work.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Schwartzberg L, Kim ES, Liu D, et al: Precision oncology: Who, how, what, when, and when not? Am Soc Clin Oncol Ed Book 37:160-169, 2017
2. Karlovich CA, Williams PM: Clinical applications of next-generation sequencing in precision oncology. Cancer J 25:264-271, 2019
3. Zeng J, Johnson A, Shufean MA, et al: Operationalization of next-generation sequencing and decision support for precision oncology. JCO Clin Cancer Inform 3:1-12, 2019
4. Ding L, Bailey MH, Porta-Pardo E, et al: Perspective on oncogenic processes at the end of the beginning of cancer genomics. Cell 173:305-320.e10, 2018
5. Beaubier N, Bontrager M, Huether R, et al: Integrated genomic profiling expands clinical options for patients with cancer. Nat Biotechnol 37:1351-1360, 2019

6. Lincoln SE, Nussbaum RL, Kurian AW, et al: Yield and utility of germline testing following tumor sequencing in patients with cancer. JAMA Netw Open 3:e2019452, 2020
7. Samadder NJ, Riegert-Johnson D, Boardman L, et al: Comparison of universal genetic testing vs guideline-directed targeted testing for patients with hereditary cancer syndrome. JAMA Oncol 7:230-237, 2020
8. Damodaran S, Berger MF, Roychowdhury S: Clinical tumor sequencing: Opportunities and challenges for precision cancer medicine. Am Soc Clin Oncol Ed Book e175-e182, 2015
9. Mosele F, Remon J, Mateo J, et al: Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: A report from the ESMO Precision Medicine Working Group. Ann Oncol 31:1491-1505, 2020
10. Mandelker D, Zhang L, Kemel Y, et al: Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. JAMA 318:825-835, 2017
11. Schrader KA, Cheng DT, Joseph V, et al: Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncol 2:104-111, 2016
12. Ceyhan-Birsoy O, Misyura M, Mandelker D: A clinical approach to detecting germline pathogenic variants from tumor-only sequencing. JNCI Cancer Spectr 4:pkaa019, 2020
13. Klek S, Heald B, Milinovich A, et al: Genetic counseling and germline testing in the era of tumor sequencing: A cohort study. JNCI Cancer Spectr 4:pkaa018, 2020
14. Mandelker D, Donoghue M, Talukdar S, et al: Germline-focussed analysis of tumour-only sequencing: Recommendations from the ESMO Precision Medicine Working Group. Ann Oncol 30:1221-1231, 2019
15. Kalia SS, Adelman K, Bale SJ, et al: Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): A policy statement of the American College of Medical Genetics and Genomics. Genet Med 19:249-255, 2017
16. Kraft IL, Godley LA: Identifying potential germline variants from sequencing hematopoietic malignancies. Hematol Am Soc Hematol Educ Program 2020:219-227, 2020
17. Garcia EP, Minkovsky A, Jia Y, et al: Validation of OncoPanel: A targeted next-generation sequencing assay for the detection of somatic variants in cancer. Arch Pathol Lab Med 141:751-758, 2017
18. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-423, 2015
19. Chakravarty D, Gao J, Phillips S, et al: OncoKB: A precision oncology knowledge base. JCO Precis Oncol 1:1-16, 2017. doi:10.1200/PO.17.00011
20. Loh JW, Guccione C, Di Clemente F, et al: All-FIT: Allele-frequency-based imputation of tumor purity from high-depth sequencing data. Bioinformatics 36:2173-2180, 2020
21. Khiabanian H, Hirshfield KM, Goldfinger M, et al: Inference of germline mutational status and evaluation of loss of heterozygosity in high-depth, tumor-only sequencing data. JCO Precis Oncol 2018, 2018. doi:10.1200/PO.17.00148
22. Khiabanian Lab: Khiabanian Lab software at Rutgers Cancer Institute, software.khiabanian-lab.org
23. PDQ® Cancer Genetics Editorial Board: Cancer Genetics Overview (PDQ(R)): Health Professional Version, PDQ Cancer Information Summaries. Bethesda, MD, National Cancer Institute, 2021
24. Stoffel EM, Carethers JM: Current approaches to germline cancer genetic testing. Annu Rev Med 71:85-102, 2020
25. Real R, Vargas JM: The probabilistic basis of Jaccard's index of similarity. Syst Biol 45:380-385, 1996
26. Yamamoto Y, Kanai M, Kou T, et al: Clinical significance of TP53 variants as possible secondary findings in tumor-only next-generation sequencing. J Hum Genet 65:125-132, 2020
27. Shiovitz S, Korde LA: Genetics of breast cancer: A topic in evolution. Ann Oncol 26:1291-1299, 2015
28. Maxwell KN, Wubbenhorst B, Wenz BM, et al: BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. Nat Commun 8:1-11, 2017
29. Lucas AL, Shakya R, Lipsyc MD, et al: High prevalence of BRCA1 and BRCA2 germline mutations with loss of heterozygosity in a series of resected pancreatic adenocarcinoma and other neoplastic lesions. Clin Cancer Res 19:3396-3403, 2013
30. Sokol ES, Pavlick D, Khiabanian H, et al: Pan-cancer analysis of BRCA1 and BRCA2 genomic alterations and their association with genomic instability as measured by genome-wide loss of heterozygosity. JCO Precis Oncol 4:442-465, 2020. doi:10.1200/po.19.00345
31. Giardiello FM, Allen JI, Axilbund JE, et al: Guidelines on genetic evaluation and management of Lynch syndrome: A consensus statement by the US Multi-Society Task Force on Colorectal Cancer. Dis Colon Rectum 57:1025-1048, 2014
32. Carlo MI, Zhang L, Mandelker D, et al: Cancer Predisposing Germline Mutations in Patients (Pts) With Urothelial Cancer (UC) of the Renal Pelvis (RP), Ureter (U) and Bladder (B). Alexandria, VA, American Society of Clinical Oncology, 2017
33. Norquist BM, Harrell MI, Brady MF, et al: Inherited mutations in women with ovarian carcinoma. JAMA Oncol 2:482-490, 2016
34. Akras Z, Bungo B, Leach BH, et al: Primer on hereditary cancer predisposition genes included within somatic next-generation sequencing panels. JCO Precis Oncol 3:1-11, 2019. doi:10.1200/PO.18.00258
35. Jonsson P, Bandlamudi C, Cheng ML, et al: Tumour lineage shapes BRCA-mediated phenotypes. Nature 571:576-579, 2019
36. AlDubayan SH, Conway JR, Camp SY, et al: Detection of pathogenic variants with germline genetic testing using deep learning vs standard methods in patients with prostate cancer and melanoma. JAMA 324:1957-1969, 2020
37. Knudson AG: Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820-823, 1971
38. Ganesan S, Garber J: Poly (ADP-ribose) polymerase inhibitor activity in prostate cancers harboring mutations in DNA repair genes: Who benefits? JCO Precis Oncol 1034-1037, 2020. doi:10.1200/PO.20.0026
39. Sun JX, He Y, Sanford E, et al: A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. PLoS Comput Biol 14:e1005965, 2018
40. Ganesan S: Tumor suppressor tolerance: Reversion mutations in BRCA1 and BRCA2 and resistance to PARP inhibitors and platinum. JCO Precision Oncol 1-4, 2018. doi:10.1200/PO.18.00001
41. Pettitt SJ, Frankum JR, Punta M, et al: Clinical BRCA1/2 reversion analysis identifies hotspot mutations and predicted neoantigens associated with therapy resistance. Cancer Discov 10:1475-1488, 2020