Cancer Genetics

ORIGINAL ARTICLE

Prevalence and characteristics of likely-somatic variants in cancer susceptibility genes among individuals who had hereditary pan-cancer panel testing

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Abstract

Next-generation sequencing (NGS) hereditary pan-cancer panel testing can identify somatic variants, which exhibit lower allele frequencies than do germline variants and may confound hereditary cancer predisposition testing. This analysis examined the prevalence and characteristics of likely-somatic variants among 348,543 individuals tested using a clinical NGS hereditary pan-cancer panel. Variants showing allele frequencies between 10% and 30% were interpreted as likely somatic and identified in 753 (0.22%) individuals. They were most frequent in *TP53, CHEK2* and *ATM*, commonly as C-to-T transitions. Among individuals who carried a likely-somatic variant and reported no personal cancer history, 54.2% (78/144) carried a variant in *TP53, CHEK2* or *ATM*. With a reported cancer history, this percentage increased to 81.1% (494/609), predominantly in *CHEK2* and *TP53*. Their presence was associated with age (OR=3.1, 95% CI 2.5, 3.7; $p<0.001$) and personal history of cancer (OR=3.3, 95% CI 2.7, 4.0; $p<0.001$), particularly ovarian cancer. Germline *ATM* pathogenic variant carriers showed significant enrichment of likely-somatic variants (OR=2.8, 95% CI 1.6, 4.9; $p=0.005$), regardless of cancer status. The appearance of likely-somatic variants is consistent with clonal hematopoiesis, possibly influenced by cancer treatment. These findings highlight the precision required of diagnostic laboratories to deliver accurate germline testing results.

Keywords   Next-generation sequencing, Hereditary pan-cancer panel testing, Somatic variant, Clonal hematopoiesis.

Introduction

Mosaicism is defined as the presence of two or more cell populations with unique genotypes in one individual. Low-level mosaicism is seen frequently in normal tissues, and particularly in those with higher turnover rates [1]. One such tissue is bone marrow, which produces the peripheral white blood cells that comprise a common source of genomic DNA used in next-generation sequencing (NGS) hereditary pan-cancer panel testing.

NGS methods enable precise assessment of variant allele proportions and thus can reveal low-level mosaicism in an individual's test sample [1,2]. Many likely-mosaic variants identified in this manner reflect amplification of mutations acquired somatically in blood via clonal hematopoiesis of indeterminate potential (CHIP), which involves the expansion of clonal blood cell subpopulations without other hematologic disease [3]. Variants derived through CHIP are not thought to represent the germline genetic profile, hematologic malignancy, post-zygotic mosaicism, circulating tumor burden, or sequencing error. Previous studies have shown that CHIP rates increase with age and with exposure to cancer treatments such as chemotherapy and radiation (cytotoxic) therapy [4–6]. Several studies have associated CHIP with increased cardiovascular disease, all-cause mortality, and susceptibility to primary and secondary leukemias (i.e., therapy related leukemia) [3,4,7–9]. Therefore, putative CHIP-derived somatic variants hold potential as independent clinical biomarkers, following clinical validation of methods for CHIP confirmation and variant classification.

Recent work demonstrated the identification of likely-somatic variants through NGS hereditary pan-cancer panel testing in a large clinical testing population [10]. The findings were consistent with previous CHIP studies, which showed likely-somatic variants occurring commonly in *TP53* (MIM: 191170) and *ATM* (MIM: 607585) and having a higher prevalence among older individuals [4,7].

Having a large NGS hereditary pan-cancer testing population presented the unique opportunity to investigate the prevalence and characteristics of likely-somatic variants in cancer susceptibility genes. A better understanding of the etiology of putative CHIP-derived somatic variants is essential to improve test reporting, patient counseling, preventive screening, and medical management. We hypothesized that cancer status (as a marker for likely cancer treatment) and/or germline mutation carrier status would contribute to the presence of likely-somatic variants. This is the first report to evaluate these factors from a hereditary cancer predisposition cohort. The analysis also examined the molecular characteristics of likely-somatic variants across the full testing population to identify trends that might provide further insight into their origins.

Methods

Testing population

The full analysis set included 348,543 individuals who had NGS hereditary pan-cancer panel testing and results reported sequentially between September 2013 and July 2017. Testing was performed by Myriad Genetics Laboratories, Inc. (Salt Lake City, UT), a national Clinical Laboratory Improvement Amendments and College of American Pathology-certified facility. All individuals provided consent for clinical testing, and testing data were de-identified for analysis. The analysis set did not include individuals who met the following criteria: cancer history listed as unspecified or colorectal polyps only on the test request form; missing age at testing; and individuals from states with laws that prevent the use of de-identified genetic data. Individuals with known leukemias are not accepted for hereditary pan-cancer panel testing by the laboratory and therefore would not have been included in the analysis set.

Sequencing and variant classification

The NGS pan-cancer panel included 28 genes commonly associated with hereditary cancer predisposition: *APC* (MIM: 611731); *ATM* (MIM: 607585); *BARD1* (MIM: 601593); *BMPR1A* (MIM: 602199); *BRCA1* (MIM: 113705); *BRCA2* (MIM: 600185); *BRIP1* (MIM: 605882); *CDH1* (MIM: 192090); *CDK4* (MIM: 123829); *CDKN2A* (p16INK4a)(MIM: 600160); *CDKN2A* (p14ARF)(MIM: 600160)*; CHEK2* (MIM: 604373); *EPCAM* (MIM: 185535); *GREM1* (MIM: 603054); *MLH1* (MIM: 120436); *MSH2* (MIM: 609309); *MSH6* (MIM: 600678); *MUTYH* (MIM: 604933); *NBN* (MIM: 602667); *PALB2* (MIM: 610355); *PMS2* (MIM: 600259); *POLD1* (MIM: 174761); *POLE* (MIM: 267800); *PTEN* (MIM: 601728); *RAD51C* (MIM: 602774); *RAD51D* (MIM: 602954); *SMAD4* (MIM: 600993); *STK11* (MIM: 602216); and *TP53*. All genes were available for the full time period except *GREM1, POLD1,* and *POLE*, which were added in July 2016. Testing was performed using blood or saliva samples. DNA extraction, testing methods, and variant identification have been described previously [11,12]. The minimum depth of coverage for sequencing was 50X. For all tested genes except *TP53*, variants present with NGS allele frequencies of 30–70% were considered to be germline. Variants with allele frequencies between 10% and 30% were considered to be likely somatic. The laboratory does not maintain data on variants with allele frequencies lower than 10%. All somatic variants were confirmed, either by Sanger sequencing or by repeat NGS analysis, when the variant allele fraction was too low to be identified by Sanger sequencing (below approximately 20%).

Variant classification was consistent with guidelines from the American College of Medical Genetics and Genomics [13] and performed as described previously [14]. For the purposes of the analyses performed here, variants with a laboratory classification of Deleterious or Suspected Deleterious were considered to be Pathogenic Variants (PV). Variants with a laboratory classification of Polymorphism or Favor Polymorphism were considered to be Benign (clinically insignificant). Variants for which the clinical significance could not be determined were classified as Variants of Uncertain Significance (VUS).

The analysis included individuals who were found to carry a likely-somatic PV or VUS in a tested cancer susceptibility gene. Individuals with an apparent germline PV in *TP53* (allele frequencies between 30% and 70%) were excluded, as it is difficult to determine whether *TP53* variants are somatic or germline in origin without follow-up testing [15]. The analysis included somatic PV and VUS in order to examine both pathogenic and potentially-pathogenic variants across the testing population. This approach provided the most complete analysis set to support the characterization of likely-somatic variants.

Statistical analyses

Multivariable logistic regression analysis was used to determine the risk of carrying a likely-somatic variant after adjusting for personal cancer history (affected or unaffected), age at testing ($<$50 versus $\geq$50 years), germline PV status (carrier or non-carrier), and clinical indication for testing. To determine the risk of carrying a likely-somatic variant, logistic regression

Table 1 Demographic characteristics of the hereditary cancer genetic testing population.

	Likely-somatic variant carriers (N=753)	Non-likely-somatic variant carriers (N=347,790)	TOTAL (N=348,543)
Age at testing			
Mean (SD)	62.6 (14.3)	48.0 (13.6)	48.0 (13.7)
Median (IQR)	64 (54, 73)	47 (38, 57)	48 (38, 58)
Min, Max	19, ≥90	11, ≥90	11, ≥90
Age at testing category			
<50 yrs.	141 (18.7%)	192,604 (55.4%)	192,745 (55.3%)
≥50 yrs.	612 (81.3%)	155,186 (44.6%)	155,798 (44.7%)
Affected status			
Affected	609 (80.9%)	146,269 (42.1%)	146,878 (42.1%)
Unaffected	144 (19.1%)	201,521 (57.9%)	201,665 (57.9%)
Ancestry			
White/non-hispanic	445 (59.1%)	169,827 (48.8%)	170,272 (48.9%)
Black/African	31 (4.1%)	23,695 (6.8%)	23,726 (6.8%)
Hispanic/Latino	26 (3.5%)	23,336 (6.7%)	23,362 (6.7%)
Asian	8 (1.1%)	8349 (2.4%)	8357 (2.4%)
Ashkenazi Jewish	12 (1.6%)	7256 (2.1%)	7268 (2.1%)
Native American	10 (1.3%)	4571 (1.3%)	4581 (1.3%)
Middle Eastern	2 (0.3%)	2378 (0.7%)	2380 (0.7%)
Pacific Islander	0	15 (<0.1%)	15 (<0.1%)
Other	8 (1.1%)	2334 (0.7%)	2342 (0.7%)
Multiple ancestries	47 (6.2%)	25,044 (7.2%)	25,091 (7.2%)
None specified	164 (21.8%)	80,985 (23.3%)	81,149 (23.3%)

SD, standard deviation; IQR, interquartile range.

analyses were performed for each tested gene separately in the subset of individuals who carried germline PVs, adjusting for personal cancer history, age at testing, and clinical indication for testing.

Chi-square tests were used to determine associations between affected status (breast/ovarian cancer vs unaffected) and type of likely-somatic variant (insertion/deletion, transversion, or transition). Odds ratios (OR), 95% confidence intervals (CI), and *p*-values are reported. Bonferroni multiple comparison *p*-value adjustments were made for the subset PV enrichment logistic regression analysis and for the breast or ovarian cancer versus variant type chi-square tests. All *p*-values of < 0.05 were considered statistically significant.

Results

Prevalence of likely-somatic variants

Demographic characteristics of the testing population, including age at testing, personal cancer history and ancestry, are shown in Table 1. From NGS analysis of the hereditary pan-cancer gene panel, 0.22% of all tested individuals (753/348,853) were found to carry one or more likely-somatic PV or VUS. Most (97.2%; 732/753) carried only one. Among individuals with likely-somatic variants, 9.0% (68/753) also carried an apparent germline PV. A total of 775 likely-somatic variants (PV and VUS) were detected in these 753 individuals. Variants were seen most commonly in *TP53* (28.9%; 224/775), *CHEK2* (28.8%; 223/775), and *ATM* (20.9%; 162/775), with a steep drop-off in frequency of variants seen in all remaining panel genes (Table 2).

Table 2 Gene distribution of likely-somatic variants (N=775).

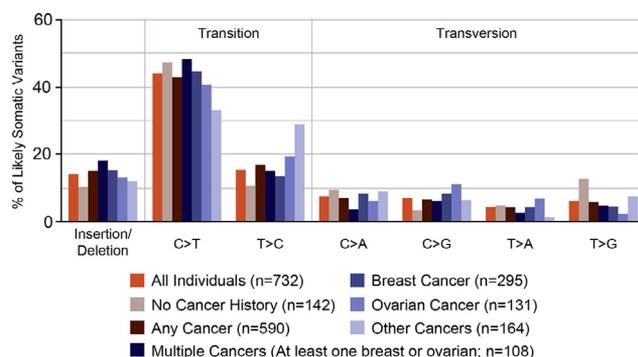| Gene	Frequency	Percent
TP53	224	28.9
CHEK2	223	28.8
ATM	162	20.9
BRCA2	23	3.0
APC	21	2.7
BRCA1	12	1.5
NBN	11	1.4
MLH1	10	1.3
PMS2	10	1.3
CDH1	9	1.2
BRIP1	8	1.0
PALB2	8	1.0
MSH6	6	0.8
BARD1	5	0.6
PTEN	5	0.6
SMAD4	5	0.6
CDK4	4	0.5
RAD51D	4	0.5
STK11	4	0.5
BMPR1A	3	0.4
CDKN2A (p14ARF)	3	0.4
MUTYH	3	0.4
POLD1	3	0.4
RAD51C	3	0.4
CDKN2A (p16INK4a)	2	0.3
MSH2	2	0.3
POLE	2	0.3
TOTAL	775	–

Fig. 1 Types of variants identified in individuals with or without personal history of cancer and a single likely-somatic variant.

Among individuals who reported no personal cancer history, 54.2% (78/144) carried a likely-somatic variant in one of these three genes. The cumulative frequency of likely-somatic variants increased to 81.1% (494/609) in individuals with a personal cancer history, most notably for *CHEK2* and *TP53*. Likely-somatic variants in *CHEK2* were seen in 16.7% (24/144) of individuals who had no cancer history and increased to 32.0% (195/609) among individuals with a cancer history. Similarly, the frequency of likely-somatic variants in *TP53* was 18.8% (27/144) in individuals who had no cancer history, increasing to 30.2% (184/609) in individuals with a cancer history. For *ATM*, the frequency remained static at 18.8% (27/144) in individuals with no cancer history compared with 18.9% (115/609) in those who reported a personal cancer history.

Characteristics of likely-somatic variants and association with cancer

A total of 593 unique likely-somatic variants were identified. Substitutions comprised 85.2% (505/593) of unique variants, and insertions or deletions comprised 14.8% (88/593). C-to-T transition variants ($C>T$; reverse strand $G>A$) were the most common, accounting for 43.5% (337/775) of all variants identified (Fig. 1). All likely-somatic variants identified in more than one individual (recurrent variants) are listed in Supplemental Table 1, with aggregate cancer type and age-at-diagnosis information for individuals with the variant who reported a personal history of cancer.

Nucleotide substitution profiles for likely-somatic variants were examined to identify potential associations with clinical factors. Similar profiles were observed for all likely-somatic variants regardless of personal cancer history (Fig. 1). Across all individuals, $C>T/G>A$ transition variants were increased compared with all other variant types. There was no significant difference in the proportions of transition and transversion mutations based on reported breast and ovarian cancer histories. The numbers of individuals who had other types of cancers and carried likely-somatic variants were too small to analyze.

Of all unique variants identified, 11.5% (68/593) were observed in more than one individual. These variants were identified in *TP53* (31), *CHEK2* (24), *ATM* (11), *CDKN2A* (p14ARF) (1) and *MLH1* (1) (Supplemental Table 1) and included five of the eight most common recurrent *TP53* variants present in the International Agency for Research on Cancer (IARC) *TP53* tumor database (c.524G$>$A [p.Arg175His]; c.742C$>$T [p.Arg248Trp]; c.743G$>$A [p.Arg248Gln]; c.818G$>$A [p.Arg273His]; and c.844C$>$T [p.Arg282Trp]) (http://p53.iarc.fr/). These "hotspot" recurrent variants were detected most commonly in CpG dinucleotides, where deamination of 5-methylcytosine is known to lead to $C>T$ transitions [16]. Recurrent variants accounted for 32.9% (241/732) of individuals carrying a single likely- somatic variant.

Age and personal history of cancer

Multivariable logistic regression analysis was performed to examine the relationships between carrying a likely-somatic variant and age or personal history of cancer (Table 3). Individuals who were 50 years of age or older at testing were found to be 3.1 times more likely to carry a likely- somatic variant compared with individuals under age 50 years (OR=3.1, 95% CI 2.5, 3.7; $p<0.001$).

A personal history of cancer was reported for 42.1% (146,878/348,543) of individuals across the entire testing population. Among individuals with one or more likely-somatic variants, a personal cancer history was reported for 80.9% (609/753). Individuals who had a personal cancer history were 3.3 times more likely to have a likely-somatic variant compared to those with no personal cancer history (OR=3.3, 95% CI 2.7, 4.0; $p<0.001$) (Table 3). This trend was most prominent in individuals with a reported personal history of ovarian cancer (1.0%; 135 with likely-somatic variants/13,777 total) as compared with individuals reporting no personal cancer history (0.1%; 144 with likely-somatic variants/201,665 total). These individuals also showed the greatest increase in the frequency of likely-somatic variants at older ages (Fig. 2). The frequency of likely-somatic variants in individuals with a reported personal history of ovarian cancer increased from 1.0% overall to 1.8% (61/3330) among individuals who were older than 71 years when tested.

Table 3 Logistic regression analysis of relationships between clinical factors and risk of carrying a likely-somatic variant, adjusting for personal cancer history, age at testing, germline pathogenic variant carrier status, and clinical indication for testing. (All tested individuals; $N=348,543$.).

Variable	Odds ratio	95% confidence limits	*p*-value
Personal cancer history(affected versus unaffected)	3.3	(2.7, 4.0)	<0.001
Age at testing(≥50 years versus <50 years)	3.1	(2.5, 3.7)	<0.001
Germline PV carrier status (positive versus negative/VUS)	1.2	(0.9, 1.5)	0.203

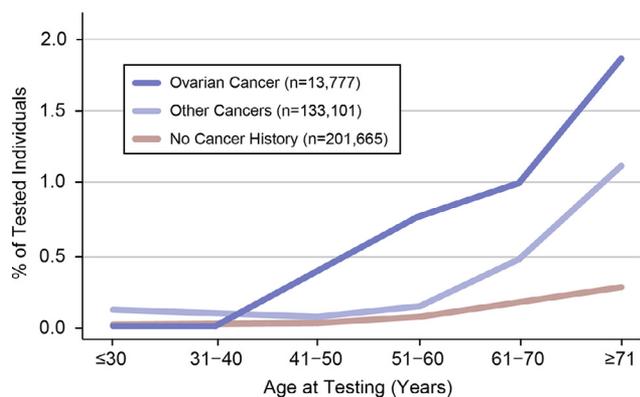PV, Pathogenic variant; VUS, Variant of uncertain significance.

Fig. 2 Percentage of individuals with a likely-somatic variant as a function of age and personal cancer history.

Germline PV carrier status

The testing population included 22,962 individuals who carried a germline PV in a cancer susceptibility gene. Logistic regression analysis showed no significant relationship between germline PV carrier status and increased likelihood of carrying a concomitant likely-somatic variant (OR=1.2, 95% CI 0.9, 1.5; $p = 0.203$) (Table 3).

To examine the relationship between germline PV carrier status and risk of carrying a likely-somatic variant in any one gene in the panel, subset analyses were performed for each gene separately. After adjusting for age at testing, personal cancer history, and clinical indication for testing, there were no significant findings associated with germline PVs in individual genes, with the exception of *ATM*. For individuals who had a single germline PV in *ATM* (211), analysis showed enrichment of likely-somatic variants compared with single non-*ATM* PV carriers (521) (OR=2.8, 95% CI 1.6, 4.9, $p = 0.005$). Interestingly, in 12 of 16 individuals who carried an apparent germline PV in *ATM*, the identified somatic variant also occurred in *ATM*. The remaining four individuals carried a somatic variant in *BRCA1* (1), *BRCA2* (1), *CHEK2* (1) and *PALB2* (1). Most individuals (75.0%; 9/12) who had a germline PV and a somatic PV or VUS in *ATM* had a personal history of cancer.

Discussion

These analyses detail the frequency and characteristics of likely-somatic variants identified within the largest hereditary pan-cancer panel testing population described to date. Collectively, likely-somatic variants were rare (0.22%). Their presence in this testing population is consistent with CHIP, given the significant associations with advanced age and with a personal history of cancer [4,7,17]. In addition, they were enriched in genes associated previously with CHIP (*ATM*, *CHEK2*, and *TP53*) [4,7,17]. Furthermore, $C > T / G > A$ transition variants were notably more frequent than were other variant types across the testing population, consistent with observations made in studies of somatic variants and age in human cancers [6,18]. The ubiquitous nature of CHIP makes this a plausible mechanism for most likely-somatic variants [4,7].

A circulating tumor burden with allele frequencies between 10–30% is a possible explanation; however, overt hematologic malignancies, when noted, were not accepted by the laboratory and therefore should not confound the analysis. As the likely-somatic variants increased with age, multi-germ layer mosaicism would be a less probable explanation for most observed variants.

The presence of likely-somatic variants was significantly associated with a personal history of cancer, possibly related to the effects of cytotoxic treatment [5,6]. The most prominent trend was seen with ovarian cancer, possibly due to the duration, amount, and/or type of chemotherapy used to treat ovarian cancer. Most individuals who have ovarian cancer undergo chemotherapy treatment, which usually involves platinum-containing compounds [19]. Therefore, of all individuals who had a reported cancer history, those with ovarian cancer might be most representative of a chemotherapy-treated population.

In this testing population, the proportion of individuals with likely-somatic *TP53* and *CHEK2* variants, but not *ATM* variants, increased among individuals with a personal history of cancer. Hematopoietic cells with a single *TP53* PV may selectively expand with exposure to cytotoxic therapy [20]. Recent analyses of somatic variants in individuals who had previous cancer treatment have produced varied results with regard to *TP53* [5,6]. Coombs et al. observed significant association of CHIP-related somatic *TP53* mutations with both chemotherapy and radiation treatment. While Swisher et al. identified somatic *TP53* mutations in individuals who received chemotherapy treatment for ovarian cancer, the association was not significant. Comparison among the current work and these recent reports is limited by differences in analysis population, cohort size, and available clinical information. The observed increase in *CHEK2* somatic variants among individuals with a personal history of cancer, similar to the increase observed for *TP53*, suggests that hematopoietic cell sub-clones harboring *CHEK2* PVs also may expand preferentially following exposure to cytotoxic therapy. The lack of a similar increase in likely-somatic *ATM* variants among individuals with a personal history of cancer indicates that hematopoietic cell sub-clones with *ATM* variants do not expand after cytotoxic therapy. These observations are consistent with the idea that haploinsufficiency of *CHEK2* and *TP53,* but not of *ATM,* drives amplification of hematopoietic cell sub-clones in individuals who have had cancer.

Unlike other studies to date, this analysis specifically examined germline hereditary cancer gene mutation status relative to the appearance of likely-somatic variants. While germline PV carrier status overall showed no significantly increased risk of carrying a likely-somatic variant, subset analysis revealed a significant association for *ATM* heterozygous germline PV carriers, independent of personal cancer history. It has been hypothesized that *ATM* germline PV carriers are more susceptible to developing clonal hematopoiesis [21]. ATM regulates the DNA damage response to double-strand DNA breaks through its kinase activity, in a pathway that also involves TP53 and CHEK2 [22]. Heterozygous germline *ATM* variants are enriched in women with breast cancer (1–2%) [12,23–25] as well as in pancreatic cancer cohorts (1.2–4.1%) [26–29]. As *ATM* germline PV carriers appear common in the population (~0.4% of non-Finnish Europeans) [23], a better understanding of the link between clonal hematopoiesis and

ATM germline PV carrier status is warranted. Notably, *ATM* germline PV carriers frequently had a likely-somatic variant in *ATM*. A recent report showed similar association of inherited *ATM* variants with acquired loss of heterozygosity at the same locus in individuals found to have mosaic chromosomal alterations presumed to arise from clonal hematopoiesis [30]. Disruption of both *ATM* alleles in some portion of an individual's blood might underpin development of chronic lymphocytic leukemia (CLL), as bi-allelic ATM inactivation is frequent in CLL [28].

The strength of this study is the large, clinical-grade data set, which provides a rare opportunity to power statistical analyses of factors associated with likely-somatic variants. At the same time, analysis of de-identified NGS clinical testing population comes with inherent limitations. First, NGS allele frequencies suggest, but cannot confirm, CHIP as the mechanistic origin of most likely-somatic variants observed in this population. Ruling out constitutional or multi-germ-layer mosaicism would require follow-up testing with additional tissues and/or family members [15], which was beyond the scope of this analysis. Second, clinical treatment details for individuals with a reported personal cancer history were unavailable, limiting the evaluation of relationships between cytotoxic therapy and the appearance of likely-somatic variants. Third, as the test was optimized to detect germline PVs, variants present at allele frequencies below 10% were not evaluated and thus were absent from reported totals. While germline testing is not optimized for somatic variant analysis, this testing pipeline provided a large and well-powered cohort in which to analyze prevalence and characteristics of likely-somatic variants, though with a variant allele frequency threshold greater than that used in other studies. Examining variants present at allele frequencies below 10% will bring needed dimension to future analyses. Finally, the analysis did not include common CHIP-associated genes, such as *DNMT3A, TET2,* and *ASXL1*, as they were not part of the clinical testing panel used here [4,7,17]. Even with these limitations, this analysis depicts a uniquely well-powered, real-world hereditary cancer risk testing scenario, further underscoring the value of laboratory precision and accurate result interpretation and reporting. Follow-up studies that include detailed cancer treatment history, variants with lower allele frequencies, and additional CHIP-associated genes will add needed dimension to the exploration of somatic variant origins.

In summary, NGS-based panel testing for hereditary cancer predisposition can identify and characterize somatic variants more readily than could previous testing methods. This work helps to highlight how frequently somatic variants can arise, likely from CHIP, with normal aging and with cancer. Several identified likely-somatic hotspot variants, particularly in *ATM, CHEK2,* and *TP53*, represent excellent candidates for future CHIP study. Somatic variant findings can meaningfully impact test result interpretation, as they may put patients at risk for CHIP-related morbidities such as primary and secondary leukemias, cardiovascular disease, and decreased overall survival [4,7,17]. Large, long-term follow-up studies are needed to better understand how somatic variants affect this spectrum of outcomes. Further, differentiating between germline and somatic acquisition of PVs in cancer-risk genes can have profound medical management implications for patients and their family members. Assay and data analysis customization, combined with follow-up confirmation using additional tissues and family member testing, is required to accurately interpret hereditary cancer predisposition test results. These findings highlight the investment that diagnostic laboratories must make to deliver a clinically accurate result.

Competing interests

BC, RB, JL, HCC, and DMD are employees of Myriad Genetic Laboratories, Inc., and receive salary and stock options as compensation. TPS, GM, JW, and SLN have no competing interests to disclose.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.04.005.

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