**Differences in Cancer Phenotypes Among Frequent CHEK2 Variants and Implications for Clinical Care—Checking CHEK2**

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**IMPORTANCE** Germline CHEK2 pathogenic variants (PVs) are frequently detected by multigene cancer panel testing (MGPT), but our understanding of PVs beyond c.1100del has been limited.

**OBJECTIVE** To compare cancer phenotypes of frequent CHEK2 PVs individually and collectively by variant type.

**DESIGN, SETTING, AND PARTICIPANTS** This retrospective cohort study was carried out in a single diagnostic testing laboratory from 2012 to 2019. Overall, 3783 participants with CHEK2 PVs identified via MGPT were included. Medical histories of cancer in participants with frequent PVs, negative MGPT (wild type), loss-of-function (LOF), and missense were compared.

**MAIN OUTCOMES AND MEASURES** Participants were stratified by CHEK2 PV type. Descriptive statistics were summarized including median (IQR) for continuous variables and proportions for categorical characteristics. Differences in age and proportions were assessed with Wilcoxon rank sum and Fisher exact tests, respectively. Frequencies, odds ratios (ORs), 95% confidence intervals were calculated, and P values were corrected for multiple comparisons where appropriate.

**RESULTS** Of the 3783 participants with CHEK2 PVs, 3473 (92%) were female and most reported White race. Breast cancer was less frequent in participants with p.I157T (OR, 0.66; 95% CI, 0.56-0.78; P < .001), p.S428F (OR, 0.59; 95% CI, 0.46-0.76; P < .001), and p.T476M (OR, 0.74; 95% CI, 0.56-0.98; P = .04) PVs compared with other PVs and an association with nonbreast cancers was not found. Following the exclusion of p.I157T, p.S428F, and p.T476M, participants with monoallelic CHEK2 PV had a younger age at first cancer diagnosis (P < .001) and were more likely to have breast (OR, 1.83; 95% CI, 1.66-2.02; P < .001), thyroid (OR, 1.63; 95% CI, 1.26-2.08; P < .001), and kidney cancer (OR, 2.57; 95% CI, 1.75-3.68; P < .001) than the wild-type cohort. Participants with a CHEK2 PV were less likely to have a diagnosis of colorectal cancer (OR, 0.62; 95% CI, 0.51-0.76; P < .001) compared with those in the wild-type cohort. There were no significant differences between frequent CHEK2 PVs and c.1100del and no differences between CHEK2 missense and LOF PVs.

**CONCLUSIONS AND RELEVANCE** CHEK2 PVs, with few exceptions (p.I157T, p.S428F, and p.T476M), were associated with similar cancer phenotypes irrespective of variant type. CHEK2 PVs were not associated with colorectal cancer, but were associated with breast, kidney, and thyroid cancers. Compared with other CHEK2 PVs, the frequent p.I157T, p.S428F, and p.T476M alleles have an attenuated association with breast cancer and were not associated with nonbreast cancers. These data may inform the genetic counseling and care of individuals with CHEK2 PVs.
The CHEK2 gene codes a protein kinase (CHK2) that acts as a tumor suppressor and plays a role in DNA damage repair.\textsuperscript{1,4} CHEK2 variants were first described among families who met clinical criteria for Li-Fraumeni syndrome (LFS) but were TP53 negative.\textsuperscript{5} CHEK2 pathogenic and likely pathogenic variants (PVs) have been associated with breast cancer (BC). Although their association with LFS has been negated,\textsuperscript{5,7} the association with other cancers remains controversial.\textsuperscript{8,10} There is an urgent need to delineate the cancer phenotypes associated with CHEK2 PVs because they are frequently identified on cancer panel testing.\textsuperscript{10,13}

The best-studied CHEK2 variant, c.1100del, is a loss-of-function (LOF) variant that has been well characterized in European populations.\textsuperscript{14-16} The cumulative risk of BC with CHEK2 c.1100del was estimated to be 37% in a meta-analysis of patients with BC.\textsuperscript{17} In the Copenhagen General Population Study,\textsuperscript{18} the c.1100del variant was associated with breast and stomach cancers and enriched in kidney cancers and sarcomas, but this was not significant after correcting for multiple comparisons. CHEK2 PVs have been associated with colorectal, kidney, prostate, and thyroid cancers,\textsuperscript{11,19-24} but these findings are limited by small sample sizes and high genetic homogeneity.

Many questions about CHEK2 and its associated cancer risks remain. In general, LOF variants tend to be PVs.\textsuperscript{25} In contrast, missense variants have more variable effects and depend on whether a critical protein domain is affected.\textsuperscript{25} A few missense variants, p.I157T, p.S428F, and p.T476M, are associated with attenuated BC risk compared with LOF PVs in CHEK2.\textsuperscript{12,26,27} Muranen et al\textsuperscript{26} compared p.I157T with c.1100del, and among patients with BC, p.I157T had a more favorable prognosis compared with those with c.1100del. Others have excluded both p.I157T and p.S428F, from analyses owing to the lower association with BC (odds ratio [OR], <1.5).\textsuperscript{12} The high population frequency of p.I157T, p.S428F, and p.T476M and the seemingly attenuated cancer risks have made it challenging to delineate CHEK2-associated cancer risks by variant type.

We aimed to compare cancer phenotypes among participants with CHEK2 PVs by variant and variant type, and to compare the cancer phenotypes of CHEK2 monoallelic and biallelic PVs.

**Methods**

This retrospective cohort study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines and was conducted to evaluate participants with CHEK2 PVs identified by genetic testing ordered by health care professionals between July 2012 through September 2019 at a single diagnostic testing laboratory (Ambry Genetics). Individuals with a concurrent PV in CHEK2 and another gene were excluded. The CHEK2 PV cohort underwent 8 to 25 gene targeted breast/ovarian cancer panel testing (n = 2199), 49 to 67 gene panel testing (n = 1857), or testing with a customizable panel of 1 to 75 genes (n = 119). The CHEK2 wild-type (WT) cohort included 33,034 participants without any PVs on a pancancer panel (49-67 genes). Individuals with variants of uncertain significance in any gene were not excluded uniformly. The Western institutional review board provided an exemption from review and a waiver of consent for the deidentified data.

Clinical characteristics, including sex, race and ethnicity, cancer history, BC hormone receptor subtype, age at genetic testing, and first cancer diagnosis were obtained from clinician-completed requisition forms and from clinical documentation (pedigrees and clinic notes) when provided. Diverse cancer types were examined including adrenal, brain, colorectal, endometrial, gastric, kidney, melanoma, ovarian, pancreatic, prostate, thyroid, and sarcoma.

Variant interpretation was performed according to a model based on the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines.\textsuperscript{28} Pathogenic and likely pathogenic variants were both denoted as PV. CHEK2 PVs were categorized as follows: biallelic, monoallelic, LOF (includes truncating, splice site variants, initiation codons, gross deletions, and duplications), missense, and more or less frequent (using an approximate 100 participant threshold). The p.I157T, p.S428F, and p.T476M variants have conflicting interpretations between laboratories in ClinVar\textsuperscript{29-32} or functional studies have led to questions about their pathogenicity.\textsuperscript{7,12,26,33-36} Therefore, the BC phenotypes of these groups were first compared with the other PV and WT CHEK2 cohorts to determine how they should be categorized for subsequent analyses.\textsuperscript{10,34}

Descriptive statistics for participants stratified by variant category are summarized as median (IQR) for continuous and proportions for categorical characteristics. Differences in ages were assessed with Wilcoxon rank sum test and proportions across variant categories were analyzed with Fisher exact tests. Frequencies of specific tumor types and ORs with 95% CIs were summarized by variant category. All statistical tests were 2-sided, and a P < .05 was considered statistically significant. Adjustments for multiple tests were made according to the Bonferroni method where applicable and P values of less than 0.00278 are significant. All analyses were conducted with R statistical software (version 4.0.4; R Foundation, Inc.).
Results

Of 36,817 evaluable participants who underwent multigene cancer panel testing, 3783 CHEK2 PVs were identified (3734 monoallelic and 49 biallelic, Figure 1). Of the 3783 individuals carrying a PV, 3473 participants (91.8%) (95% CI, 90.9%-92.7%) were female, 2818 (74.5%) had cancer (95% CI, 73.1%-75.9%), and, of the female participants, 2202 (63.4%) (95% CI, 61.7%-65.0%) had BC. The most frequent monoallelic PV was c.1100del (n = 1252) followed by p.I157T (n = 992), p.S428F (n = 324), p.T476M (n = 250), p.R117G (n = 125), exon 8_9 deletion (ex8_9del, n = 113), and c.444 + 1G>A (n = 92) (Figure 1). The ex8_9del variant is a recurrent 5395 base pair deletion that spans coding exons 8 and 9.37

Breast Cancer Among Frequent PVs

We compared the BC prevalence among participants with the 7 more frequent PVs to all other CHEK2 PVs and to CHEK2 WT to inform our classification of the proposed lower-risk variants in our cohort. The BC prevalence was highest among participants with c.444+1G>A (OR, 2.63; 95% CI, 1.59-4.35; P < .001), ex8_9del variants (OR, 2.36; 95% CI, 1.53-3.64; P < .001), followed by p.R117G (OR, 1.65; 95% CI, 1.12-2.44; P = .01) and c.1100del (OR, 1.76; 95% CI, 1.55-2.00; P < .001; Figure 2, eTable 8 in the Supplement) compared with WT.

The BC prevalence was lower with the p.I157T, p.S428F, and p.T476M PVs compared with other PVs (P < .001) and the ORs were fewer than 1.4 compared with WT (Figure 2; eTables 1-3 in the Supplement). Owing to these marked differences from other PVs, p.I157T, p.S428F, and p.T476M were deemed lower risk and analyzed and reported separately.

Cancer Phenotypes of Participants With CHEK2 PVs and WT

More participants reported White race (1707 [78.7%]) among CHEK2 PVs compared with the WT cohort (21907 [66.3%]) (Table; eTable 2 in the Supplement). Cancer characteristics were compared between the PV cohort (n = 2168 after excluding p.I157T, p.S428F, and p.T476M) and the WT cohort. Participants with PVs were more likely to be diagnosed with a cancer (1664 [76.8%]) compared with WT (23,065 [69.8%]) (OR, 1.43; 95% CI, 1.29-1.58; P < .001, Table). Median (IQR) age at first cancer diagnosis was younger among participants with PVs: 47 (41-56) vs 49 (43-59) years for WT (Table). A history of multiple primary tumors, defined as a primary cancer of more than 1 site (eg, breast and colon), was more frequent among the PV cohort than WT (OR, 1.37; 95% CI, 1.18-1.58; P < .001, Table).

The CHEK2 PV cohort was more likely to have a personal history of BC (n = 1339, 67.1%) than WT (n = 16,029, 52.7%) with an OR of 1.83 (95% CI, 1.66-2.02; P < .001). The median (IQR) age at first BC diagnosis was slightly younger among the PV cohort (47 [41-56] years) compared with WT (50 [43-59] years, Table). Other significant differences in BC were noted among the PV cohort vs the WT cohort, with the former including more bilateral BCs (OR, 1.99; 95% CI, 1.74-2.28; P < .001), more estrogen and/or progesterone receptor (ER/PR)-positive BCs (OR, 3.64; 95% CI, 2.81-4.78; P < .001), and more ERBB2 (formerly HER2) positive BCs (OR, 1.88; 95% CI, 1.26-2.82; P < .001).
HER2-positive BC (OR, 1.49; 95% CI, 1.23-1.79; \( P < .001 \); Table 1).

Nonbreast Cancers Among Participants
With CHEK2 PVs and WT
In addition to BC, the CHEK2 PV cohort had more kidney (OR, 2.57; 95% CI, 1.75-3.68; \( P < .001 \)) and thyroid cancers (OR, 1.63; 95% CI, 1.26-2.08; \( P < .001 \)) compared with WT (Table 1). The PV cohort had less colorectal cancer (CRC) than those in the WT group, 5.0% vs 7.8% (OR, 0.62; 95% CI, 0.51-0.76; \( P < .001 \); Table 1).

Both endometrial cancer (2.1% vs 4.2%, \( P < .001 \)) and pancreatic cancer (0.9% vs 1.8%, \( P < .001 \)) were less frequent in the PV cohort than those in the WT cohort (Table 1; eTable 4 in the Supplement). The frequencies of brain, gastric, melanoma, sarcoma, prostate, and ovarian cancer were not different between the 2 cohorts (Table 1; eTable 4 in the Supplement).

Frequent CHEK2 PVs and Cancer Phenotype
The individual frequent PVs, p.R117G, c.444+1G>A, c.1100del, and ex8,9del were compared with the other CHEK2 PVs and WT by cancer type. Participant BC frequencies were overlapping for different CHEK2 PVs and higher than those in the WT cohort: p.R117G (OR, 1.65; 95% CI, 1.12-2.44; \( P = .01 \)); p.I157F (OR, 2.20; 95% CI, 1.31-3.69; \( P = .02 \)) and c.1100del (OR, 1.97; 95% CI, 1.65-2.34; \( P < .001 \)) PVs compared with those in the WT group (eTable 8 in the Supplement).

Compared with WT, the c.1100del PV was associated with kidney (OR, 3.11; 95% CI, 1.96-4.74; \( P < .001 \)) and thyroid cancer (OR, 1.68; 95% CI, 1.21-2.3; \( P = .002 \); eTable 8 in the Supplement). In contrast, CRC (OR, 0.69; 95% CI, 0.53-0.88; \( P = .002 \); eTable 8 in the Supplement) and endometrial cancer (OR, 0.46; 95% CI, 0.29-0.69; \( P < .001 \); eTable 5 in the Supplement) were less frequent in participants with CHEK2 c.1100del than those in the WT cohort. Other examined cancers (adrenal, brain, gastric, sarcoma, prostate, and ovarian cancer) were not associated with CHEK2 PV (eTable 5 in the Supplement).

Biallelic CHEK2, LOF, Missense, and c.1100del
Comparisons were conducted for biallelic (n = 21) vs monoallelic (n = 2168) (eTable 6 in the Supplement); LOF (n = 1924) vs missense (n = 244) variants (eTable 7 in the Supplement); and c.1100del (n = 1252) vs other PVs (n = 916) for any cancers and BC (eTable 8 in the Supplement, Figure 3).

The biallelic CHEK2 PV cohort had a younger age at any cancer diagnosis (median age, 37 years vs 47 years; \( P < .001 \)) and at BC diagnosis (median age, 40 years vs 47 years; \( P = .02 \)) compared with the monoallelic PV cohort (Figure 3; eTable 6 in the Supplement). Breast cancer was more frequent in the biallelic cohort compared to the WT group and this was statistically significant (\( P < .001 \)).

There were no significant differences in age at cancer diagnosis between LOF vs missense variants and c.1100del vs other PVs. OR indicates odds ratio; PV, pathogenic or likely pathogenic variant.
Table. Cohort Characteristics and Cancer Associations Stratified by Wild-type vs Monoallelic Pathogenic Variants

| Characteristic                          | No. (%)                        | CHEK2 monoallelic PVa OR (95% CI) | P value |
|----------------------------------------|--------------------------------|----------------------------------|---------|
| **Sex**                                |                                |                                  |         |
| Female                                 | 30 429 (92.1)                  | 1995 (92.0)                      | 0.99 (0.85-1.17) | .93     |
| Male                                   | 2605 (7.9)                     | 172 (7.9)                       | NA      | <.001   |
| **Age, median (IQR), y**               |                                |                                  |         |
| At genetic testing                     | 53 (44-63)                     | 53 (43-62)                      | NA      | .005    |
| At first breast cancer                 | 50 (43-59)                     | 47 (41-56)                      | NA      | <.001   |
| At first cancer                        | 49 (42-58)                     | 47 (40-56)                      | NA      | <.001   |
| **Race and ethnicity, ancestry**       |                                |                                  |         |
| African American/Black                 | 1815 (5.5)                     | 37 (1.7)                        | 0.26 (0.18-0.36) | <.001   |
| Ashkenazi Jewish                       | 2247 (6.8)                     | 92 (4.2)                        | 0.53 (0.42-0.65) | <.001   |
| Asian                                  | 1071 (3.2)                     | 20 (0.9)                        | 0.24 (0.15-0.37) | <.001   |
| Hispanic                               | 1582 (4.8)                     | 66 (3.0)                        | 0.54 (0.41-0.69) | <.001   |
| White                                  | 21 907 (66.3)                  | 1707 (78.7)                     | 0.72 (0.62-0.82) | <.001   |
| Other                                  | 4409 (13.4)                    | 246 (11.4)                      | 1.43 (1.29-1.58) | <.001   |
| **Any cancer**                         |                                |                                  |         |
| Yes                                    | 23 065 (69.8)                  | 1664 (76.8)                     | NA      | NA      |
| No                                     | 9969 (30.2)                    | 504 (23.3)                      | NA      | NA      |
| **Primary cancers, No.**               |                                |                                  |         |
| 0                                      | 9969 (30.2)                    | 504 (23.3)                      | NA      | <.001   |
| 1                                      | 18 498 (56.0)                  | 1348 (62.2)                     | NA      | <.001   |
| >1                                     | 4567 (13.8)                    | 316 (14.6)                      | NA      |         |
| **Breast cancer**                      |                                |                                  |         |
| Yes                                    | 16 029 (52.7)                  | 1339 (67.1)                     | 1.83 (1.66-2.02) | <.001   |
| No                                     | 13 584 (44.6)                  | 620 (31.1)                      | NA      |         |
| **Bilateral breast cancer**            |                                |                                  |         |
| Yes                                    | 2228 (7.3)                     | 273 (13.7)                      | 1.99 (1.74-2.28) | <.001   |
| No                                     | 27 345 (89.9)                  | 1683 (84.4)                     | NA      |         |
| **First breast cancer ER/PR positive** |                                |                                  |         |
| Yes                                    | 8355 (78.0)                    | 837 (92.8)                      | 3.64 (2.81-4.78) | <.001   |
| No                                     | 2362 (22.0)                    | 65 (7.2)                        | NA      |         |
| **First breast cancer ERBB2 positive** |                                |                                  |         |
| Yes                                    | 1492 (19.0)                    | 168 (25.9)                      | 1.49 (1.23-1.79) | <.001   |
| No                                     | 6362 (81.0)                    | 482 (74.2)                      | NA      |         |
| **Colorectal cancer**                  |                                |                                  |         |
| Yes                                    | 2575 (7.8)                     | 109 (5.0)                       | 0.62 (0.51-0.76) | <.001   |
| No                                     | 29 550 (89.5)                  | 2015 (92.9)                     | NA      |         |
| **Kidney cancer**                      |                                |                                  |         |
| Yes                                    | 214 (0.7)                      | 36 (1.7)                        | 2.57 (1.75-3.68) | <.001   |
| No                                     | 31 896 (96.6)                  | 2088 (96.3)                     | NA      |         |
| **Melanoma**                           |                                |                                  |         |
| Yes                                    | 1259 (3.8)                     | 60 (2.8)                        | 0.71 (0.54-0.93) | .01d    |
| No                                     | 30 854 (93.4)                  | 2064 (95.2)                     | NA      |         |
| **Pancreatic cancer**                  |                                |                                  |         |
| Yes                                    | 597 (1.8)                      | 20 (0.9)                        | 0.5 (0.3-0.78) | <.001   |
| No                                     | 31 517 (95.4)                  | 2104 (97.1)                     | NA      |         |
| **Thyroid cancer**                     |                                |                                  |         |
| Yes                                    | 696 (2.1)                      | 74 (3.4)                        | 1.63 (1.26-2.08) | <.001   |
| No                                     | 31 416 (95.1)                  | 2050 (94.6)                     | NA      |         |

Abbreviations: ER, estrogen receptor; ERBB2, formerly HER2 (human epidermal growth factor receptor 2); NA, not applicable; PR, progesterone receptor; PV, pathogenic or likely pathogenic variant; WT, wild type.

a Excluding the lower risk CHEK2 variants: p.I157T, p.S428F, and p.T476M.
b Sex was not available for 1 participant.
c ER/PR positive breast cancer included tumors that were estrogen receptor positive and/or progesterone receptor positive.
d After Bonferroni correction, this P value was not significant.
other PVs for both any cancer types including BC (Figure 3; eTables 7 and 8 in the Supplement).

**Frequent Lower-Risk CHEK2 Variants**

The lower-risk variants, p.I157T, p.S428F, and p.T476M were examined separately by comparing cancer characteristics of each group with the WT and the PV cohorts (eTables 1-3 in the Supplement).

There were no significant differences in the frequencies of any cancer including BC between the p.I157T and WT cohorts (eTable 1 in the Supplement). The frequency of bilateral BC (OR, 1.52; 95% CI, 1.23-1.89; \( P = .001 \)) was higher with p.I157T than those in the WT cohort. However, when compared with the other PVs, the p.I157T cohort had a significantly lower frequency of BC (OR, 0.66; 95% CI, 0.56-0.78; \( P < .001 \); eTable 1 in the Supplement). When comparing p.I157T with WT and with other PVs, there were no differences in the frequencies of any cancer diagnosis including colorectal, kidney, and thyroid cancer.

Compared with WT, the p.S428F cohort was no different in the frequencies of any cancer diagnosis, BC, and bilateral BCs (eTable 2 in the Supplement). Any cancer (OR, 0.50; 95% CI, 0.39-0.65; \( P < .001 \)), BC (OR, 0.59; 95% CI, 0.46-0.76; \( P < .001 \)), and bilateral BC (OR, 0.44; 95% CI, 0.27-0.44; \( P < .001 \)) were less frequent in the p.S428F cohort compared with other PVs.

The frequency of BC was higher with p.T476M compared with WT (OR, 1.35; 95% CI, 1.03-1.77; \( P = .03 \)), but lower compared with other PVs (OR, 0.74; 95% CI, 0.56-0.98; \( P = .04 \)) (eTable 3 in the Supplement), though neither was significant when correcting for multiple comparisons. There were no significant differences for any cancer, bilateral BC, CRC, kidney, and thyroid cancers between p.T476M, other CHEK2 PVs, and WT (eTable 3 in the Supplement).

**Discussion**

Germline CHEK2 PVs are frequently identified on cancer panel testing and are enriched in female participants with BC.38,39 Except for the frequent lower-risk variants (p.I157T, p.T476M, and p.S428F), we found that most CHEK2 PVs, whether missense or LOF, were associated with similar cancer phenotypes. This has important implications for counseling and care of individuals with lower-risk variants because they accounted for 41.9% of the total CHEK2 monoallelic PV cohort. There were no differences in BC prevalence or age at onset when comparing LOF vs missense CHEK2 PVs.

Collectively, CHEK2 PVs were associated with BC (OR, 1.83), ER/PR-positive BC (OR, 3.60), ERBB2 protein–positive BC (OR, 1.50), and bilateral BC (OR, 2.00) compared with WT. These findings confirm previous associations of CHEK2 c.1100del with BC47,40,41 and bilateral BC.52 As in other studies, we found CHEK2 PVs to be associated with ER/PR-positive and ERBB2 protein–positive BC.12,25,40,42-44

Currently, patients with CHEK2 PVs initiate BC screening at age 40 with magnetic resonance imaging (MRI) and mammogram, and earlier if there is a family history of early-onset BC.6 A recent comparative modeling study45 proposed annual breast MRI beginning at age 30 to 35 years with the addition of mammogram at age 40 years for women with CHEK2 PVs. We found the median (IQR) age of first BC diagnosis was 47 (41-50) years, which suggests screening prior to 35 years may be of limited utility.

Biallelic CHEK2 PVs were associated with higher rates of BC and earlier age of BC (median age, 40 years) compared with monoallelic PVs (median age, 47 years), a finding consistent with Rainville et al.46 As compared with monoallelic PVs, biallelic CHEK2 PVs were also associated with an even earlier age
of any cancer by a decade (median age, 37 vs 47 years for monoallelic PVs). This finding is noteworthy and supports even earlier screening for patients with biallelic CHEK2 PVs.

CHEK2 PVs were associated with multiple primary (P < .001), kidney (OR, 2.6; P < .001) and thyroid cancers (OR, 1.6; P < .001) compared with WT. An association with kidney cancer was previously suggested for c.1100del (hazard ratio, 3.61; P = .01), although not statistically significant after Bonferroni correction. In the present study, the association remains significant even when correcting for multiple comparisons, likely due to the larger sample size. This association is further supported by studies of patients with kidney cancers in which CHEK2 PVs were overrepresented and found in 2%-3.5% of patients. More data on the prognosis of CHEK2-associated kidney cancers including modeling and cost-effectiveness studies are needed to inform screening.

An association with thyroid cancer was suggested by Naslund-Koch et al., though it, too, was not significant after Bonferroni correction. Here, we found a persistent association supporting the work of Kamihara et al, who found CHEK2 PVs were enriched among patients with thyroid cancer. As above, more data are needed on the aggressiveness of CHEK2-associated thyroid cancers to inform surveillance.

CHEK2 PVs were not associated with colorectal cancer compared with WT (OR, 0.62). These results are consistent with a meta-analysis on CHEK2 and CRC, and the Copenhagen study, where the control group was population-based and negative for c.1100del. In totality, these findings suggest intensive CRC screening may not be indicated and present guidelines for the care of patients with CHEK2 PVs should be refined.

Consistent with prior literature, the p.I157T and p.S428F variants were less frequently associated with BC compared with other CHEK2 PVs. When corrected for multiple comparisons, BC frequency with p.T476M was not different from other PVs or from WT. Moreover, p.I157T, p.T476M, and p.S428F were associated with lower rates of non-BCs compared with WT. Classification of these variants is inconsistent, and in a previous study of bioinformaticists and clinical geneticists, Agaoglu et al showed that the p.T476M alteration was classified as a variant of unknown significance, likely pathogenic or pathogenic by different interpreters. Although BC frequency with the p.I157T variant was not significantly different from WT, the frequency of bilateral BCs was (OR, 1.52; P < .001), indicating this variant may act in concert with other genetic modifiers influencing BC risk.

In 2019 the American Society of Breast Surgeons recommended all women with BC consider germline genetic testing. As testing expands, more patients will be identified with the lower-risk CHEK2 PVs (p.I157T, p.S428F, and p.T447M), hastening the need for health care professionals to be knowledgeable and to counsel patients about CHEK2 cancer associations (or lack thereof) prior to making BC surgical decisions.

Limitations

Limitations of this study include a homogenous (predominantly White female participants) study population, most with a history of BC. Recognizing that men are less likely to undergo genetic testing, there are ascertainment biases inherent in a cohort selected for genetic testing for cancer predisposition. Ordinarily, these ascertainment biases would lead to overestimations in the ORs for cancers. However, our WT cohort was subject to the same testing bias, which may lower ORs for cancer for CHEK2 PVs. Therefore, these data were not used to estimate cancer risk by genotype as this cohort was not population based.

Conversely, some tumor associations may not be apparent, such as those previously reported for cancers of the colorectum and prostate, owing to this bias. In a recent phenome-wide association study, CHEK2 was associated with leukemia and plasma cell neoplasms; however, this was not evaluable in our study because peripheral blood is not the optimal specimen for germline testing of patients with hematologic malignant diseases. We did not evaluate the frequency of nonmelanoma skin cancer despite evidence for an association. Single-nucleotide variation testing and polygenic risk scores were unavailable to further refine genotype and phenotype. The large number of participants with the lower-risk alleles, p.I157T, p.S428F, and p.T476M, allowed us to examine these separately. However, it is possible that some less frequent alleles included in the PV category may also be associated with attenuated phenotypes.

These data provide keen insights into genotype-phenotype associations for CHEK2. CHEK2 PVs were associated with a younger age at any cancer diagnosis, with multiple primary cancers; ER/PR-positive, ERBB2 protein–positive BC, bilateral BC, and kidney and thyroid cancers. Other previously reported associations, including CRC, were not identified. This large study discerns cancer phenotype by genotype. Genetic modifiers affect CHEK2 penetrance and in the future will likely aid in cancer risk stratification of patients with CHEK2 PVs. Although additional studies in population-based cohorts are needed, these data help to refine cancer associations of CHEK2 PVs and lower-risk alleles.

Conclusions

In this cohort study CHEK2 PVs, except the low-penetrance alleles of p.I157T, p.S428F, and p.T476M, were associated with similar cancer phenotypes irrespective of variant type (missense or LOF). Associations with ERBB2-positive BC, kidney, and thyroid cancers were noted while associations with CRC and other cancers were not found. These data may inform genetic counseling and risk assessment of patients with CHEK2 PVs.
Differences in Cancer Phenotypes Among Frequent CHEK2 Variants and Implications for Clinical Care

Concept and design: Bychkovsky, Agaoglu, Horton, Young, Garber, Rana. Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Bychkovsky, Agaoglu, Yussuf, Rana. Critical revision of the manuscript for important intellectual content: Bychkovsky, Agaoglu, Horton, Zhou, Hemyari, Richardson, Young, LaDuca, McGuinness, Scheib, Garber, Rana. Statistical analysis: Bychkovsky, Agaoglu, Zhou, Hemyari, Richardson, Young, Rana. Obtained funding: Rana. Administrative, technical, or material support: Horton, Yussuf, Richardson, Young, LaDuca, Rana. Supervision: Bychkovsky, Hemyari, Richardson, Scheib, Garber, Rana. Other - patient advocate: McGuinness. Other - clinical relevance in breast oncology: Scheib.

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