




## ARTICLE

# Cancer burden in individuals with single versus double pathogenic variants in cancer susceptibility genes



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## ABSTRACT

**Purpose:** As panel testing expands, more individuals with double pathogenic variants (DPVs) in cancer susceptibility genes are likely to be identified. Little is known about the effects of DPVs on cancer phenotype, although this information is crucial for genetic counseling and risk management. We sought to describe the cancer phenotype among individuals with DPVs in cancer susceptibility genes.

**Methods:** A retrospective study of individuals with DPVs identified through a single testing laboratory from 2012 to 2017 was conducted. DPV combinations were enumerated. For DPV gene combinations that occurred >10 times, cancer histories of individuals with DPVs were compared with cancer histories of controls with a single PV matched by gene.

**Results:** Among 644 individuals with DPVs, combinations that included the *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2* genes occurred >10 times. There were 8883 matched controls for a single PV in these genes. The median age of first cancer diagnosis was younger with *ATM+CHEK2* (43), compared with *ATM* (47,  $P = .016$ ) or *CHEK2* (47,  $P = .015$ ) alone. Similar findings were observed when comparing age at first breast cancer (BC) for the *ATM+CHEK2* with single-gene controls. Individuals with 2 *CHEK2* PVs also were younger at first cancer diagnosis (40) compared with single *CHEK2* PV controls (47,  $P = .0038$ ). This difference was not driven by age at first BC diagnosis among females.

**Conclusion:** Individuals with *ATM+CHEK2* or 2 *CHEK2* PVs have a greater cancer burden than single gene controls. These findings can be used to counsel individuals with DPVs and their families and inform cancer screening recommendations.

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## Introduction

A personal or family history of cancer is an indication for germline genetic testing of cancer susceptibility genes. This testing is often completed through multigene panel testing (MGPT). MGPT has the potential to identify pathogenic or likely pathogenic variants (PVs) in different genes. Although having multiple monogenic hereditary cancer syndromes is not common, the finding can be alarming for individuals and providers alike because it raises many questions about cancer risk and management. The presence of double pathogenic variants (DPVs) in one individual may affect cancer phenotype, age at cancer diagnosis or tumor subtype, which is important for genetic counseling and clinical care. However, because its rarity, studies on individuals with DPVs have been limited by sample size or to a small subset of genes.

Prior studies have found that 1% to 2% of individuals tested for hereditary cancer have >1 PV; however, those with >2 PVs are less frequent.<sup>1-6</sup> Most studies have focused on individuals with both *BRCA1* and *BRCA2* PVs, previously referred to as double heterozygosity.<sup>7</sup> Individuals with both *BRCA1* and *BRCA2* PVs (*BRCA1+BRCA2*) were more likely to be diagnosed with breast cancer (BC) than individuals with either a *BRCA1* or *BRCA2* PV alone.<sup>7</sup> Although the age of BC diagnosis was similar to individuals with a *BRCA1* PV, individuals with PVs in both *BRCA1* and *BRCA2* had more estrogen receptor (ER)-positive BCs than individuals with *BRCA1* PVs alone.<sup>7</sup>

There is less known about the effects of DPVs in other genes. In 1 study of 11 individuals with DPVs, almost all had malignancies associated with their respective PVs.<sup>2</sup> However, there is a lack of understanding of the tumor biology or epistatic effects of DPVs because most in vivo models of hereditary cancer syndromes have focused on characterizing PVs in a single cancer susceptibility gene.<sup>8</sup>

Herein, we describe the landscape of DPVs identified on MGPT and enumerate the most frequent DPVs gene combinations. We delineate the phenotype of individuals with DPVs by gene combinations, sex, cancer type, and age of diagnosis for BC and first cancer. We compare cancer histories of individuals with DPVs with controls with a single PV matched by gene. For females with DPVs associated with BC and a personal history of BC, we report the hormone receptor (HR) subtype. The goal of this work is to provide information for genetic counseling of individuals with DPVs.

## Materials and Methods

A retrospective study was conducted of individuals who were tested by MGPT for personal or family history of cancers between March 2012 and March 2017 on panels of varying sizes (5-67 genes) at a single diagnostic laboratory. A full gene list and NCBI reference numbers are provided in

the supplement (Supplemental Table 1). Patients with monoallelic PVs in genes associated predominantly with autosomal recessive or biallelic cancer predisposition syndromes (*BLM*, *FANCC*, *MUTYH*, *RAD50*, and *XRCC2*) were excluded from this analysis entirely because of limited evidence of their cancer associations in the heterozygous state. The *CHEK2* p.I157T, p.S428F, and p.T476M variants and *APC* p.I1307K were defined as lower-risk PVs and separated from other PVs.<sup>9,10</sup> Cases with more than 2 PVs were also excluded from the study. Individuals with biallelic PVs in genes associated with autosomal recessive cancer predisposition were only included if they also had an additional PV in another gene (eg, biallelic *MUTYH* and *BRCA2*). Based on the most frequent DPV combinations, control cohorts were selected, including individuals with a single PV in *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2*. The control cohorts were tested on a 67-gene panel, and controls were selected if they had a PV in the gene of interest and were negative for PVs in the other 66 genes. The 67-gene panel covers all the genes on the smaller panels used for this study.

Clinical characteristics, including cancer history, sex, and age at testing for each tumor diagnosis, were obtained from test requisition forms and clinical documentation, including pedigrees and chart notes, when provided. Variant interpretation was performed according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines.<sup>11</sup> Pathogenic and likely pathogenic variants were denoted as PVs in this study. When DPVs were identified in the same gene, phase was categorized as likely/confirmed in trans, likely/confirmed in cis, or phase unknown. Likely/confirmed trans was applied to PVs in the same gene that are recurrent and are known to occur on different haplotypes (likely) or when trans phase was molecularly confirmed (confirmed).<sup>12</sup>

Descriptive statistics for individuals stratified by DPV combination were summarized, and included sex, median age of first cancer (interquartile range [IQR]), proportion and 95% confidence intervals (CI) of female individuals with BC, individuals with non-BCs, individuals with more than 1 cancer diagnosis, and reported BC HR subtype. Individuals were sorted by their DPVs and analyzed when >10 had the same combination (eg, *ATM+BRCA2*, *BRCA1+CHEK2*). Comparisons were made with single-PV controls matched by gene using *t* tests and Wilcoxon rank sum, when applicable, for median age at BC diagnosis in female individuals and median age at first cancer diagnosis and using Fisher's exact test for number of primary tumors (>2 cancers). All statistical tests were two-sided. With exception of the *CHEK2+CHEK2* gene combination, adjustments for multiple tests were made according to the Bonferroni method and a *P* value of less than .025 was considered statistically significant. Because *CHEK2+CHEK2* was compared with only monoallelic *CHEK2*, *P* value of less than .05 was considered statistically significant. All analyses were conducted with R v.4.0.4.

## Results

### Cohort demographics

Among 653 individuals with multiple PVs (MPVs), 544 (83.3%) (95% CI, 80.3%-86.0%) were female, 440 (67.4%) (95% CI, 63.7%-70.9%) were White, and the median age of testing was 53 (IQR: 19; Table 1). Most individuals with MPVs had a reported cancer diagnosis: 52.8% (95% CI, 49.0%-56.6%) had a single cancer diagnosis and 27.9% (95% CI, 24.6%-31.4%) had >2 cancer diagnoses, whereas 117 (17.9%) (95% CI, 15.2%-21.0%) were unaffected by cancer at the time of genetic testing. Among the 8883 individuals serving as matched controls for *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2*, most were female (93.0%) (95% CI, 92.4%-93.5%), White (64.7%) (95% CI, 63.7%-65.7%), and had a personal history of cancer (79.6%) (95% CI, 78.8%-80.4%). Among the controls, the median age of genetic testing was 52 (IQR: 19), similar to those with DPVs.

### Distribution of DPVs

We excluded 9 (1.3%) individuals with >2 PVs from the analysis: 5 with 3 PVs, 3 with >2 lower-risk PV, and 1 with 1 PV and 2 lower-risk PVs. Of the remaining 644 individuals with DPVs, 157 (24.3%) had 1 PV with 1 lower-risk PV, and 26 individuals (4%) had 2 lower-risk PVs only (Figure 1). There were 461 cases (71.6%) with DPVs in moderate- or high-penetrance cancer susceptibility genes. Of these, 405 (62.3%) individuals had PVs in different genes, and 56 (8.7%) had PVs in the same gene. Among individuals with PVs in the same gene, 18 had 2 PVs in *CHEK2*: 9 were confirmed trans and 9 were likely trans because they were combinations of founder PVs that have been described independently (eg, c.1100del and c.444+1G>A; Figure 1).

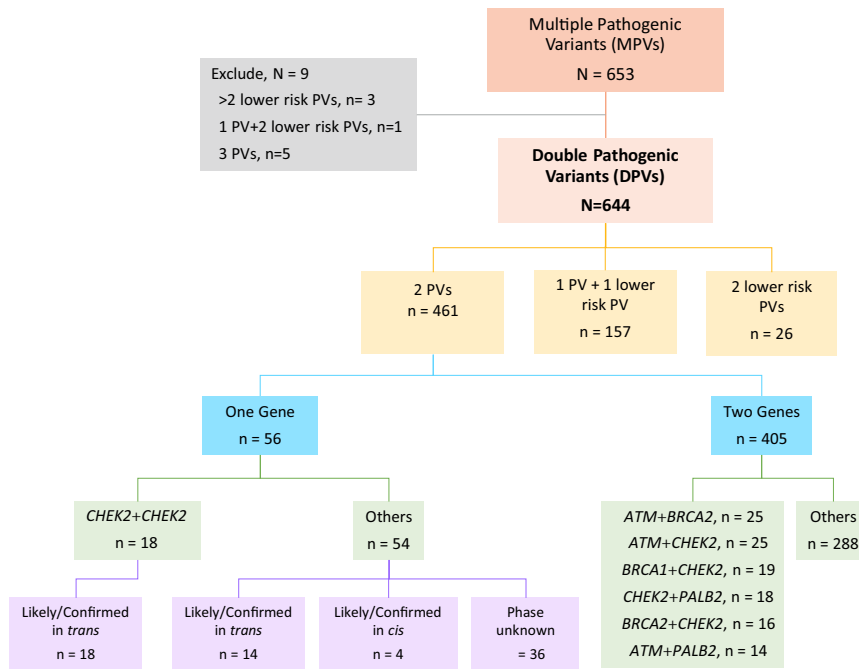
The most frequent recurrent DPVs were enumerated, and cancer histories are reported (Figure 2, Table 2). The combination of DPVs in *ATM+CHEK2* ( $n = 25$ ) and *ATM+BRCA2* ( $n = 25$ ) were equally frequent. *CHEK2* in combination with *BRCA1* ( $n = 19$ ), *CHEK2* ( $n = 18$ ),

**Table 1** Characteristics of MPV cohort and single-gene controls

Sample Characteristic	MPVs	Single-PV Controls					Total Controls N (%)
	N (%)	<i>ATM</i> N (%)	<i>BRCA1</i> N (%)	<i>BRCA2</i> N (%)	<i>CHEK2</i> N (%)	<i>PALB2</i> N (%)	
Total N	653	1367 (15.4)	2535 (28.5)	2668 (30)	1605 (18.1)	708 (8)	8883 (100)
Sex							
Female	544 (83.3)	1275 (93.3)	2407 (95.0)	2425 (90.9)	1475 (91.9)	676 (95.5)	8258 (93.0)
Male	109 (16.7)	92 (6.7)	128 (5.0)	243 (9.1)	130 (8.1)	32 (4.5)	625 (7.0)
Race, ethnicity, and ancestry							
African American/Black	19 (2.9)	58 (4.2)	228 (9.0)	234 (8.8)	24 (1.5)	45 (6.4)	589 (6.6)
Ashkenazi Jewish	59 (9.0)	74 (5.4)	185 (7.3)	146 (5.5)	67 (4.2)	21 (3.0)	493 (5.5)
Asian	14 (2.1)	36 (2.6)	127 (5.0)	136 (5.1)	16 (1.0)	35 (4.9)	350 (3.9)
Hispanic	25 (3.8)	63 (4.6)	223 (8.8)	180 (6.7)	45 (2.8)	46 (6.5)	557 (6.3)
White	440 (67.4)	936 (68.5)	1433 (56.5)	1604 (60.1)	1299 (80.9)	475 (67.1)	5747 (64.7)
Other/Unknown	96 (14.7)	200 (14.6)	339 (13.4)	368 (13.7)	154 (9.6)	86 (12.1)	1147 (12.9)
Number of primary cancer(s)							
0	117 (17.9)	271 (19.8)	430 (17.0)	465 (17.4)	316 (19.7)	107 (15.1)	<sup>a</sup>
1	345 (52.8)	762 (55.7)	1542 (60.8)	1621 (60.8)	895 (55.8)	448 (63.3)	<sup>a</sup>
≥2	182 (27.9)	299 (21.9)	490 (19.3)	506 (19.0)	365 (22.7)	144 (20.3)	<sup>a</sup>
Unknown	9 (1.4)	35 (2.6)	73 (2.9)	76 (2.8)	29 (1.8)	9 (1.3)	<sup>a</sup>
Median age at genetic testing (IQR)	53 (19)	52 (19)	49 (20)	53 (21)	53 (20)	53 (19)	52 (19)
Median age at first cancer diagnosis (IQR)	46 (16)	47 (17)	44 (16)	49 (18)	47 (15)	48 (16)	<sup>a</sup>
Breast cancer among females							
Yes	321 (59.0)	832 (65.3)	1483 (61.6)	1529 (63.1)	1023 (69.4)	524 (77.5)	<sup>a</sup>
No	215 (39.5)	410 (32.2)	854 (35.5)	829 (34.2)	428 (29.0)	143 (21.2)	<sup>a</sup>
Unknown	8 (1.5)	33 (2.6)	70 (2.9)	67 (2.8)	24 (1.6)	9 (1.3)	<sup>a</sup>
Median age at first breast cancer diagnosis (IQR) among females	47 (16)	46 (15)	41 (15)	46 (16)	47 (15)	48 (15)	<sup>a</sup>

IQR, interquartile range; MPV, multiple PVs in one individual (adjective); MPVs, multiple PVs in one individual (plural noun); PV, pathogenic or likely pathogenic variant.

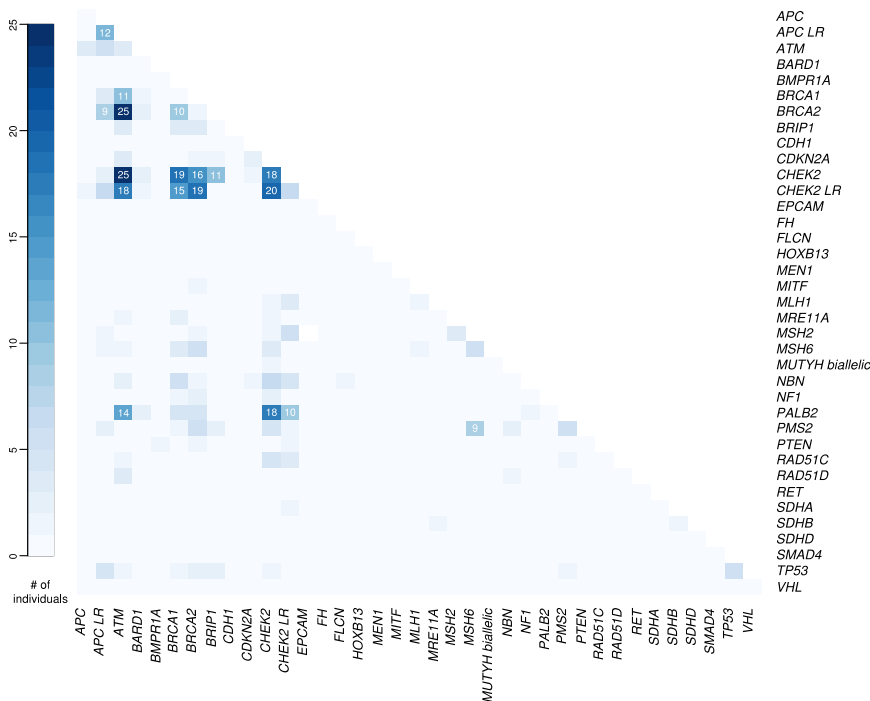
<sup>a</sup>Not analyzed because of heterogeneity.



**Figure 1 Flow Diagram of DPV Cohort.** DPVs, double pathogenic or likely pathogenic variants in one individual (plural noun); MPVs, multiple pathogenic or likely pathogenic variants in one individual (plural noun); PV, pathogenic or likely pathogenic variant (single noun); PVs, pathogenic or likely pathogenic variants (plural noun).

*PALB2* ( $n = 18$ ), and *BRCA2* ( $n = 16$ ) was the next most frequent finding. All individuals with the combination of *CHEK2+CHEK2* PVs were females, most had BC (94.4%)

and multiple primary tumors (61.1%). PVs in *BRCA1* in combination with *BRCA2* were detected in 10 individuals (Figure 2, Table 2).



**Figure 2 Heatmap of DPV Gene Combinations.**

**Table 2** Cancer phenotype of individuals with DPVs by gene combination

DPVs	Total N/F/M	N with Cancer F (% of Females)/ M (% of Males)	N of ≥2 Primary Cancer F (%) / M (%)	Median (IQR) Age of 1st Cancer	N of Female BC (%) <sup>a</sup>	Median (IQR) Age of Female BC	N of all Cancer Excluding Female BC <sup>b</sup> (%)
<i>ATM+BRCA2</i>	25/19/6	14 (56.0) 4 (16.0)	4 (16.0) 1 (4.0)	49.5 (19.5)	11 (57.9)	44 (14)	2 Male BC (33.3 of M) 1 PC (16.7 of M) 3 OV (15.8 of F) 1 CRC (4.0) 1 Mel (4.0) 1 TC (4.0) 1 CC (4.3)
<i>ATM+CHEK2</i>	25/23/2	22 (88.0) 1 (4.0)	6 (24.0) 0 (0.0)	43 (10.5)	20 (87.0)	42.5 (9.5)	1 OV (4.3 of F) 1 VulC (4.3 of F) 1 KidC (4.0) 1 Male BC (50.0 of M) 1 PC (50.0 of M) 2 OV (11.8 of F) 2 EC (11.8 of F) 1 LC (5.3) 1 Mel (5.3)
<i>BRCA1+CHEK2</i>	19/17/2	10 (52.6) 2 (10.5)	4 (21.1) 0 (0.0)	44 (10.5)	9 (52.9)	45 (9)	3 TC (16.7) 2 BlC (11.1) 2 CRC (11.1) 2 LK (11.1) 1 Mel (5.6) 1 OV (5.6 of F) 1 SarC (5.6) 1 SIC (5.6) 1 EC (5.6 of F)
<i>CHEK2+CHEK2</i>	18/18/0	17 (94.4) 0 (0.0)	11 (61.1) 0 (0.0)	40 (15)	17 (94.4)	45.5 (11)	1 OV (5.6 of F) 1 TC (5.6) 1 SIC (5.6) 1 LK (5.6)
<i>CHEK2+PALB2</i>	18/18/0	17 (94.4) 0 (0.0)	5 (27.8) 0 (0.0)	47 (18)	17 (94.4)	47 (18)	1 OV (6.7 of F) 1 LP (6.3)
<i>BRCA2+CHEK2</i>	16/15/1	11 (68.8) 0 (0.0)	2 (12.5) 0 (0.0)	45 (13)	10 (66.7)	43.5 (8.75)	3 OV (25.0 of F) 1 EC (8.3 of F) 1 PaC (7.1)
<i>ATM+PALB2</i>	14/12/2	11 (78.6) 1 (7.1)	4 (28.6) 0 (0.0)	51.5 (11)	9 (75.0)	49 (10)	1 PC (100.0 of M) 4 OV (40.0 of F) 2 TC (18.2) 1 EC (10.0 of F) 1 GC (9.1) 1 KidC (9.1)
<i>ATM+BRCA1</i>	11/10/1	9 (81.8) 1 (9.1)	3 (27.3) 1 (9.1)	44 (13)	4 (40.0)	39.5 (7.25)	

(continued)

Table 2 Continued

DPVs	Total		N with Cancer		N of $\geq 2$ Primary Cancer		Median (IQR)		N of all Cancer Excluding Female BC <sup>a,b</sup> (%)	
	N/F/M	F (% of Females)/M (% of Males)	F (%)	M (%)	F (%)	M (%)	Age of 1st Cancer	Age of Female BC	Female BC <sup>a,b</sup> (%)	Female BC <sup>a,b</sup> (%)
BRCA1+BRCA2	10/9/1	7 (70.0) 1 (10.0)	1 (10.0)	0 (0.0)	39.5 (18.25)	5 (55.6)	33 (11)	2 OV (22.2 of F) 1 CRC (10.0)		

Counts and percentages of males are italicized.

BC, breast cancer; BiC, bladder cancer; CC, cervical cancer; CRC, colorectal cancer; DPVs, double pathogenic or likely pathogenic variants in 1 individual (plural noun); EC, endometrial cancer; GC, gastric cancer; KidC, kidney cancer; LC, lung cancer; LK, leukemia; LP, Lymphoma; Mel, melanoma; OV, ovarian cancer; PaC, pancreatic cancer; PC, prostate cancer; SarC, sarcoma; SiC, small intestine cancer; TC, thyroid cancer; VulC, vulvar cancer.

<sup>a</sup>The percentage represents those among females.

<sup>b</sup>The percentage for OV, EC, CC, and VulC represents those among females. The percentage for PC and male BC represents those among males.

## Cancer histories of frequent DPV combinations compared with matched controls

For each of the above gene combinations, the median age of first BC diagnosis among female individuals was also compared with single-PV controls matched by gene (*ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2*, Figure 3A). The median age of first BC diagnosis was younger for individuals with combined *ATM+CHEK2* (42.5, IQR: 9.5) than it was for controls with PVs in either *ATM* (46, IQR: 15;  $P = .01$ , statistically significant after Bonferroni correction) or *CHEK2* (47, IQR: 15;  $P = .0046$ , statistically significant after Bonferroni correction). Among the 9 female individuals with PVs in *BRCA1+BRCA2*, the median age at BC diagnosis was 33 (IQR: 11), which was significantly younger than age at BC diagnosis among the *BRCA2* control cohort (46, IQR: 16;  $P = .02$ , statistically significant after Bonferroni correction), but not statistically significantly different from the *BRCA1* control cohort (41, IQR: 15;  $P = .1$ ). Differences in median age at first BC were not different between individuals with 2 *CHEK2* PVs (45.5) compared with those with a single *CHEK2* PV (47, IQR: 16;  $P = .15$ ). Other gene combinations (*ATM+BRCA1*, *ATM+BRCA2*, *ATM+PALB2*, *BRCA1+CHEK2*, *BRCA1+PALB2*, *BRCA2+CHEK2*, *BRCA2+PALB2*, *CHEK2+PALB2*) did not differ in median age of first BC diagnosis compared with each of their respective matched single-PV controls (Figure 3A).

The median age of first cancer diagnosis was also compared by DPV combination with matched controls (Figure 3B, Table 2). The median age of first cancer diagnosis was younger for individuals with *ATM+CHEK2* (43, IQR: 10.5) compared with *ATM* alone (47, IQR:17;  $P = .016$ , statistically significant after Bonferroni correction) and compared with *CHEK2* alone (47, IQR:15;  $P = .015$ , statistically significant after Bonferroni correction). Individuals with 2 *CHEK2* PVs also had a younger age first cancer diagnosis (40, IQR: 15) compared with single *CHEK2* PV controls (47, IQR: 15;  $P = .0038$ ). Although the median age of first cancer diagnosis for individuals with PVs in *BRCA1+BRCA2* (39.5) was younger than *BRCA1* (44, IQR:16) and *BRCA2* (49, IQR:18), these differences were not statistically significant. Likewise, the other examined gene combinations (*ATM+BRCA1*, *ATM+BRCA2*, *ATM+PALB2*, *BRCA1+CHEK2*, *BRCA1+PALB2*, *BRCA2+CHEK2*, *BRCA2+PALB2*, *CHEK2+PALB2*) were not associated with younger age of first cancer compared with their respective gene matched controls (Figure 3B). The most frequent gene combination among male individuals was *ATM+BRCA2* ( $n = 6$ ), too few to make meaningful comparisons with sex-matched controls.

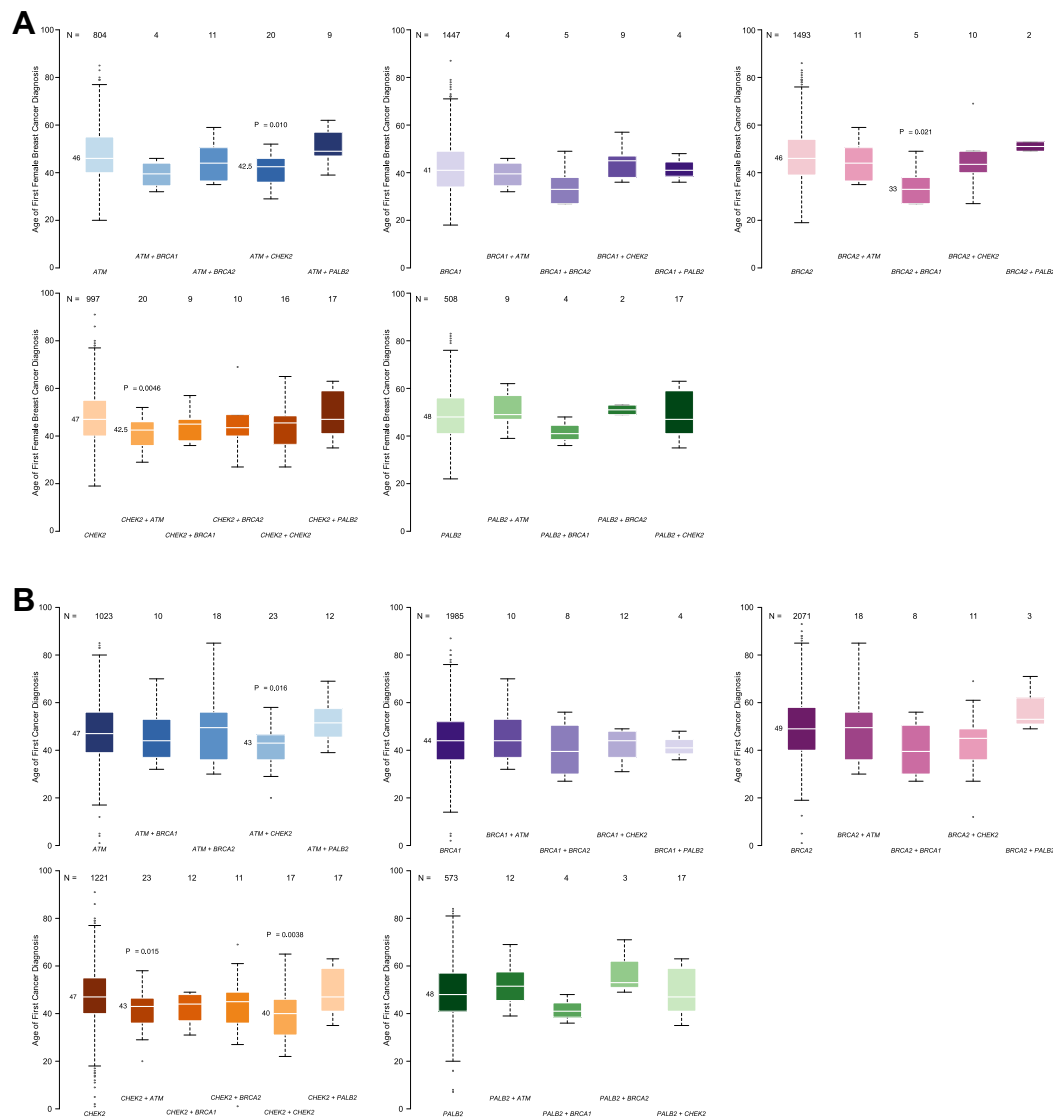
The number of primary tumors was also assessed in individuals with the most frequent DPV combinations compared with matched single-PV controls (Supplemental Table 2). Individuals with combined *CHEK2+CHEK2* PVs were more likely to have multiple primary cancers ( $>2$ ) compared with individuals with a single *CHEK2* PV (OR

5.21; 95% CI.83-15.97;  $P < .001$ ). Other DPV gene combinations did not demonstrate statistically significant differences in the number of individuals with  $\geq 2$  primaries compared with matched single-PV controls.

### Breast cancer HR subtype

Among the 544 females with DPVs, 59.0% (321) had a reported BC diagnosis (Table 1). Of the 321 BC cases in females, HR subtype was reported for 65.4% (210/321, Supplemental Table 3), and HER2 receptor status was reported for 51.7% (166/321, Supplemental Table 3). Most

BC cases were ER- and/or progesterone receptor (PR)-positive (ER/PR+) 76.7% (161/210), and 22.38% were HER2-positive (37/166). ER/PR+ BC was the most common HR subtype reported with PVs in *CHEK2+CHEK2* (100.0% among the 12 BCs with ER/PR status provided), *ATM+CHEK2* (93.3%, 14/15), *BRCA1+CHEK2* (85.7%, 6/7), and *BRCA2+CHEK2* (85.7%, 6/7; Supplemental Table 4). HER2 status was available for 11 breast tumors in *CHEK2+PALB2* heterozygotes and none was HER2-positive. Among DPVs, 34 cases were triple negative (Supplemental Table 3), 16 of whom had a *BRCA1* PV in combination with another PV.



**Figure 3** A. Median age of first breast cancer (BC) diagnosis. With exception of the *CHEK2+CHEK2* combination,  $P$  values are statistically significant after the Bonferroni correction if  $P < .025$ . When *CHEK2+CHEK2* was compared with monoallelic *CHEK2*, the  $P$  value is statistically significant if  $P < .05$ . B. Median age of first cancer diagnosis. With exception of the *CHEK2+CHEK2* combination,  $P$  values are statistically significant after the Bonferroni correction if  $P < .025$ . When *CHEK2+CHEK2* was compared with monoallelic *CHEK2*, the  $P$  value is statistically significant if  $P < .05$ .

## Discussion

The likelihood of identifying DPVs in a patient is increasing because of the widespread use of multigene panel testing for cancer susceptibility.<sup>1–5</sup> However, there have been little data on the cancer phenotypes of individuals with DPVs. This study provides insights on the frequency of DPVs and more frequent PV gene combinations while also delineating the associated cancer histories compared with control cohorts matched by gene.

Overall, among individuals with DPVs, the median ages of BC diagnosis and first cancer diagnosis were similar to individuals with a single matched PV, with a few notable exceptions. First was the combination of DPVs in *ATM+CHEK2*. Here, PVs in 2 moderate risk genes were associated with younger age BC diagnosis than having a single PV in either moderate risk gene (*ATM* or *CHEK2*) alone. The statistically significant difference in the age of cancer diagnosis with *ATM+CHEK2* compared with a single PV in *ATM* or *CHEK2* alone is almost entirely attributable to the difference in the age of BC diagnosis.

Next, the median age at BC diagnosis with PVs in *BRCA1+BRCA2* (33), although younger than the median age at diagnosis in *BRCA1* controls (41), was not statistically different. When compared with median age at BC diagnosis among *BRCA2* controls (46), BC was diagnosed over a decade earlier, and this was statistically significant. Further investigation is needed to elucidate whether the differences observed are because of the presence of a *BRCA1* variant or if differences are due to the added PV load. Among the females with PVs *BRCA1+BRCA2* and BC, 55.5% (5/9) had ER/PR+ tumors compared with 23.5% of tumors with *BRCA1* and 50.8% of tumors with *BRCA2*. These findings inform clinical management and are similar to prior work from the CIMBA consortium.<sup>7</sup> In that report, there were significantly more ER+ BCs (42.9%) with *BRCA1+BRCA2* than a single *BRCA1* PV (24.0%) and significantly fewer ER+ BCs than with a single *BRCA2* PV (76.5%), suggesting that the *BRCA1+BRCA2* combination influences tumor biology.<sup>7</sup> In this context, it is unclear if chemoprevention with tamoxifen, raloxifene, or an aromatase inhibitor would affect the BC risk in individuals with the combination of *BRCA1+BRCA2*. Another focused family analysis indicated that individuals with both *BRCA1+BRCA2* PVs had higher cancer incidence than individuals with PVs in either gene alone and surmised this group might derive greater benefit from more intensive screening or risk-reducing interventions.<sup>13</sup>

Biallelic (confirmed or likely trans phase) *CHEK2* was associated with younger ages at first cancer diagnosis compared with a single PV in *CHEK2*. Although median age at first BC was also younger for biallelic *CHEK2*, this was not statistically significant. However, a significantly younger age of BC diagnosis among biallelic *CHEK2* has previously been reported by our group and others in a larger

cohort.<sup>9,14</sup> The median age of first cancer diagnosis for *CHEK2+CHEK2* was younger than median age of first BC—suggesting that females with *CHEK2+CHEK2* have a risk of a non-BC at a younger age than BC. Moreover, individuals with biallelic *CHEK2* PVs were more likely to have  $\geq 2$  primary cancers compared with single *CHEK2* PV heterozygotes. These findings suggest that genetic counseling and management of cancer risks with biallelic *CHEK2*, when confirmed in trans, are distinct from the identification of a single *CHEK2* PV alone. Individuals with multiple primary cancers have a high rate of a PVs in cancer susceptibility genes<sup>15</sup>; however, it is unknown whether individuals with DPVs are more likely to have multiple primary cancers than individuals with a single PV.

Unexpectedly, most BCs that occurred in the context of PVs in *BRCA1+CHEK2* were also ER/PR+ (86%). This finding should be validated in large, independent data sets. Potential epistatic effects of DPVs will need to be studied in vivo before making definitive assertions regarding biological impact or claims of synergistic cancer risks.

Finally, prior work has indicated that *CHEK2*-associated breast tumors are more likely to be HER2-positive than those in the general population. In this cohort, there was a higher portion of HER2 positive tumors with the *CHEK2+CHEK2* (40%) and *BRCA1+CHEK2* (29%) combinations compared with *CHEK2* alone (26%); there were no HER2-positive tumors with the *CHEK2+PALB2* combination (0/11). Further study of these differences could be important to better understanding the pathogenesis of HER2+ BC.

Limitations of this study include a highly selected testing cohort, predominantly comprised of White female individuals with a personal history of BC. One challenge in the field of cancer genetics is that historically there has been an emphasis on hereditary cancer genetic testing for females with BC, and although guidelines for testing have broadened, testing among males remains underutilized.<sup>16–20</sup> Most individuals had germline genetic testing because of their personal diagnosis of BC; thus, DPV gene combinations relating to non-BC susceptibility syndromes were infrequent. There were too few males to allow for meaningful comparisons of the cancer phenotype or age at cancer onset among males with DPVs and the single-PV controls as we did for BC among females. To mitigate the effects of testing bias, phenotype comparisons were made to matched controls with a single PV in from the same testing cohort and therefore subject to the same testing bias as subjects with DPVs. Another limitation comes from the nature of the study. Because germline DPVs are rare, we were limited by small numbers of recurrent DPV combinations for phenotype analysis. To address this, we restricted our analysis to DPV combinations that occurred in  $>10$  subjects. Our analyses excluded individuals with DPVs that had a lower-risk pathogenic variant, namely, the *CHEK2* p.I157T, p.S428F, and p.T476M variants<sup>9</sup> and *APC* I1307K,<sup>10</sup> although the



combination of 1 PV and a lower-risk PV comprised 24.0% (157/653) of the overall cohort. Among the 156 patients included in our detailed phenotype analysis, 14 (8.9%) had the 9-gene panel; thus, it is possible (1.4% probability based on our findings in this study) that they could have a third pathogenic variant potentially altering their overall cancer burden. Notably, we focused on breast cancer phenotype and most high-risk breast cancer genes are included in small panels.

The approach to individuals with DPVs in cancer susceptibility genes are multifold. Genetic counseling should be prioritized and cascade testing of family members performed to inform cancer risks. Because medical management of DPVs in different genes can be nuanced, it is critical to evaluate whether the associated cancer risks are overlapping (eg, each PV is associated with BC susceptibility), whether there are additional, mutually exclusive risks (eg, 1 PV associated with BC and 1 PV associated with colorectal cancer), or whether there is a combination of overlapping and additional risks. More data and additional functional studies are needed to make accurate predictions about the role of DPVs in cancer development or progression. Until we better understand the implications of DPVs on cancer pathogenesis and risk, assumptions about synergistic effects of double PVs should not be made as they are not necessarily predictable.

Notable findings from this work include that age of cancer onset is younger among individuals with *ATM+CHEK2* and *CHEK2+CHEK2*. We also confirmed prior work showing age at BC onset with *BRCA1+BRCA2* is similar to *BRCA1* alone, yet with a HR subtype that mimics *BRCA2*. Exploratory analyses suggest certain combinations of PV in BC susceptibility genes may be associated with unusual and unexpected BC HR subtypes. Further studies are needed to characterize the age-specific cancer risks and tumor subtypes with DPVs to inform screening and preventative care.

## Data Availability

All data generated or analyzed during this study have been de-identified and are included in this published article.

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## Ethics Declaration

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Western institutional review board and an exemption for a waiver of consent for the de-identified data was granted.

## Conflict of Interest

Carolyn Horton, Min-Tzu Lo, Dr Polfus, Cassidy Carraway, Parichehr Hemyari, and Colin Young report employment at Ambry Genetics, a commercial lab. Min-Tzu Lo reports current employment at Monogram Biosciences. Judy E. Garber reports research collaboration with Ambry Genetics and Invitae (no compensation). Huma Q. Rana reports research collaboration with Invitae and Ambry Genetics (no compensation). All other authors declare no conflicts of interest.

## Additional Information

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