MULTIPLEX IDENTIFICATION OF GENETIC ETIOLOGIES AMONG WOMEN WITH BILATERAL BREAST CANCER USING A 25-GENE HEREDITARY CANCER PANEL

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BACKGROUND

- A hallmark of hereditary cancer predisposition is multiple primary cancers within an individual.
- Technical advances in sequencing and identification of additional cancer susceptibility genes have led to multi-gene panel approaches to determine if patient cancers have a heritable cause.
- Multiplex testing of multiple breast cancer associated genes to determine the prevalence, spectrum and combinations of mutations has not yet been evaluated in a large set of patients with two primary breast cancers.

OBJECTIVE

- The aim of this analysis was to examine the spectrum of pathogenic variants and clinical characteristics for individuals with two breast cancers who underwent testing with a 25-gene hereditary cancer panel.

METHODS

- Individuals with two breast cancer diagnoses were identified from 135,609 consecutive cases that underwent a 25-gene hereditary cancer panel test at a commercial diagnostic laboratory.
- The 25-gene panel included *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, SMAD4, STK11, RAD51C, RAD51D* and *TP53*.
- Sequencing and large rearrangement was performed for all the genes in the panel (large rearrangement only for *EPCAM*).
- Pathogenic variants (PVs) are those that received a laboratory classification of Deleterious or Suspected Deleterious.
- Clinical information was obtained by healthcare provider report on test requisition forms.
- 270 individuals with three or more breast cancers were excluded. Individuals with DCIS were included.
- Pearson's chi-square tests were performed to determine a difference between single or dual breast cancer status, synchronous diagnosis status, age of first diagnosis across mutation status, and multiple mutation status across single or dual breast cancer status. A p-value <0.05 was considered statistically significant.

RESULTS

- Among the 135,609 tested individuals, 38,440 had a single breast cancer diagnosis and 4,845 were diagnosed with two primary breast cancers.
- 12.4% (n = 603) of individuals with two breast cancers had at least one PV.
  - This is significantly higher than the 9.1% PV prevalence in individuals with one breast cancer (p < 0.0001).
- 92.9% of PVs identified in individuals with two breast cancers occurred in genes associated with an increased risk of breast cancer (Table 1).

Table 1. Distribution of PVs in Individuals with Two Breast Cancers*

Gene	Count	% of Mutations
Genes Associated with Breast Cancer		
BRCA1	168	26.9%
BRCA2	140	22.4%
CHEK2	79	12.7%
PALB2	67	10.7%
ATM	64	10.3%
BARD1	15	2.4%
BRIP1	13	2.1%
NBN	13	2.1%
TP53	12	1.9%
PTEN	5	0.8%
CDH1	4	0.6%
STK11	0	0
Total	**580**	**92.9%**
Genes Associated with Other Cancers		
MSH6	12	1.9%
PMS2	10	1.6%
RAD51C	8	1.3%
MSH2	4	0.6%
RAD51D	4	0.6%
APC	3	0.5%
CDKN2A	1	0.2%
EPCAM	1	0.2%
MLH1	1	0.2%
MUTYH	0	0
SMAD4	0	0
Total	**44**	**7.1%**
TOTAL	**624**	

*Includes individuals with >1 PV

- The remaining 7.1% of PVs were in genes associated with other cancer risks.
- The median age of diagnosis for individuals with a PV and two breast cancers was 45 years, compared to 49 years old for those without a PV.
- Individuals with two breast cancers were statistically more likely to have a PV if the first diagnosis occurred before age 45 (p < 0.0001).
- 22% (range 18-35%) of individuals whose first breast cancer was diagnosed ≤ 40 years of age had a PV (Figure 1, Table 2).
- 10% (range 8-12%) of individuals diagnosed >40 years of age had a PV; this rate persisted despite increasing age at diagnosis.

Figure 1. The proportion of individuals with two breast cancers (A) with a PV and (B) tested, according to age at first diagnosis.

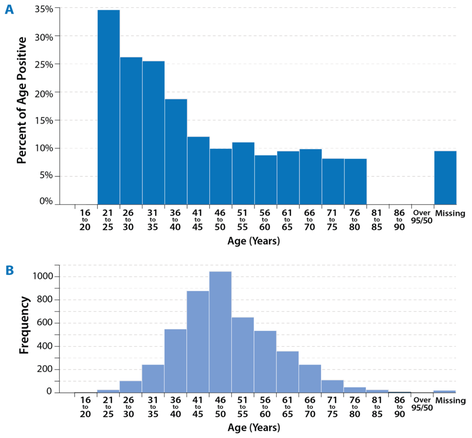

Table 2. Age of First Diagnosis

Age	All Patients		Positive Patients	
	Frequency	Percent (of All)	Frequency	Percent (of Age)
16 – 20	2	<0.1	0	0
21 – 25	26	0.5	9	34.6
26 – 30	103	2.1	27	26.2
31 – 35	243	5.0	62	25.5
36 – 40	549	11.3	103	18.8
41 – 45	878	18.1	106	12.1
46 – 50	1045	21.6	104	10.0
51 – 55	650	13.4	72	11.1
56 – 60	535	11.0	47	8.8
61 – 65	358	7.4	34	9.5
66 – 70	243	5.0	24	9.9
71 – 75	110	2.3	9	8.2
76 – 80	49	1.0	4	8.2
81 – 85	26	0.5	0	0
86 – 90	6	0.1	0	0
>90	1	<0.1	0	0
Missing	21	0.4	2	9.5

- There were significantly more PVs among individuals with metachronous disease (14.1%) than among those with synchronous breast cancers (9.7%) (p < 0.0001).
  - This may be explained, in part, by the younger median age at first diagnosis for metachronous (45 years) versus synchronous (48 years) breast cancers.
  - The prevalence of PVs was >10% for individuals with metachronous breast cancers, regardless of time between diagnoses (Figure 2).
- 20/4,845 (0.4%) of individuals with two breast cancers were found to have more than one PV (Table 3).
- This is significantly higher than the 88/38,440 (0.2%) of individuals with a single breast cancer found to have more than one PV (p = 0.0156).
  - The most common combination of PVs in individuals with two breast cancers was *CHEK2* and *PALB2* (n=6), representing 30% of the total; this combination only represented 6% of cases with 2 PVs among women with one breast cancer.

Figure 2. The proportion of individuals with two breast cancers with a PV, according to time between first and second diagnosis.

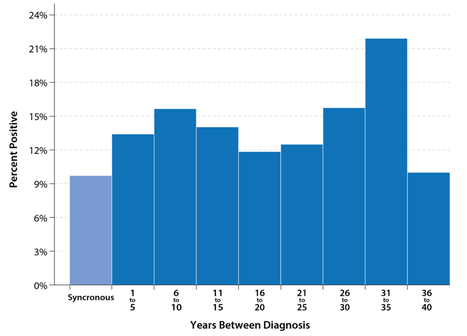

Table 3. Individuals with Two PVs

Gene	2 BC	1 BC	Gene	2 BC	1 BC
APC, BRCA1	1	0	*BRCA2, CHEK2*	0	6
ATM, BRCA1	0	5	*BRCA2, MLH1*	0	2
ATM, BRCA2	2	5	*BRCA2, MUTYH*	0	1
ATM, BRIP1	0	2	*BRCA2, NBN*	1	1
ATM, CHEK2	0	3	*BRCA2, PALB2*	0	2
ATM, PALB2	0	2	*BRCA2, PMS2*	0	4
ATM, RAD51C	0	1	*BRCA2, RAD51D*	1	0
BARD1, BRCA1	0	1	*BRCA2, SMAD4*	0	1
BARD1, BRCA2	0	1	*BRIP1, NBN*	0	1
BARD1, NBN	0	1	*CDH1, CHEK2*	1	1
BARD1, PMS2	0	1	*CHEK2, MSH6*	0	1
BRCA1, BRCA2	2	6	*CHEK2, PALB2*	6	5
BRCA1, BRIP1	2*	6	*CHEK2, RAD51C*	1	0
BRCA1, CHEK2	1	6	*CHEK2, RAD51D*	0	1
BRCA1, MSH2	0	2	*EPCAM, PALB2*	0	1
BRCA1, MSH6	0	1	*MLH1, RAD51C*	0	1
BRCA1, NBN	0	2	*MSH2, NBN*	0	1
BRCA1, PALB2	1	4	*MSH6, PMS2*	1	0
BRCA1, PMS2	0	2	*NBN, PMS2*	0	1
BRCA1, RAD51D	0	1	*P16, PALB2*	0	1
BRCA1, TP53	0	1	*PALB2, PMS2*	0	2
BRCA2, BRIP1	0	1	*PALB2, TP53*	0	1
			TOTAL	**20**	**88**

*One individual was found to have mutations in *BRCA1*, *BRIP1*, and *NBN*

CONCLUSION

- Multiplex testing in women with two primary breast cancers identifies a relatively high percentage with a PV, including those whose first diagnosis was after 50 years of age.
- Women with two primary breast cancers were twice as likely to have more than one PV compared to those with a single breast cancer.
- Double *CHEK2/PALB2* PVs were the most frequent combination among women with two primary breast cancers; 5 times more frequent than among women with one breast cancer, suggesting a possible synergistic or additive effect.
- This study adds to our understanding of breast cancer susceptibility, and reaffirms that multiple primary breast cancers is an important prompt for genetic testing.
- It is important to identify those with heritable cancer syndromes at their first diagnosis, given that 65% of cases had metachronous tumors.

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