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Cancer Predisposition Genes in metastatic breast cancer – Association with metastatic pattern, prognosis, patient and tumor characteristics

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BACKGROUND

New treatment strategies for metastatic breast cancer (mBC) are mainly driven by therapies against specific targets. BRCA mutations are one of the few established actionable targets, with PARP-Inhibitors and Platinum showing high efficacy in mBC. Hereditary cancer testing panels are now broadly used for identification of individuals with *BRCA1/2* mutations who may benefit from these therapies. Many of these panels also contain other predisposition genes involved in BRCA-related DNA repair pathways, but the clinical relevance of mutations in these genes remain unclear. The aim of this study was to describe the mutation rates of *BRCA1/2* and panel-based predisposition genes, and the associated clinical characteristics of individuals with these mutations, in a prospective cohort of mBC patients.

STUDY DESIGN AND METHODS

The PRAEGNANT mBC registry (NCT02338167) is a prospective registry for metastatic breast cancer patients with a focus on molecular biomarkers. Patients receiving any therapeutic regimen are eligible for this registry. Germline DNA was collected at study entry and genotyped for 37 cancer predisposition genes including *BRCA1* and *BRCA2*. The frequency of mutations in the *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *ATM*, *RAD51D*, *BARD1* and *MSH6* moderate and high risk genes was determined, and associations between mutations and patient and tumor characteristics, metastatic pattern, and overall survival were assessed.

Data collection

Clinical data were collected by trained and dedicated staff at the sites participating in the prospective PRAEGNANT study. These data are monitored using automated plausibility checks and through random on-site field monitoring.

Blood Samples and Genotyping

Germline DNA was extracted from EDTA-Blood samples, which were obtained at the time of study entry. DNA libraries for coding sequences and intron/exon boundaries of coding exons from 37 cancer risk genes, including *BRCA1* and *BRCA2,* were generated using a QIAseq multiplex PCR-base panel. Libraries were pooled in batches of 768 and sequenced on a HiSeq4000 to 400× mean coverage of target regions. After demultiplexing, trimming of adapters, and alignment of sequences to the human genome, variants were called using GATK Haplotype Caller and Vardict. Likely pathogenic mutations were validated using Sanger sequencing. Copy number variants were identified using PatternCNV.

Statistical Analysis

Continuous characteristics are presented as means and standard deviations (SD), categorical characteristics are presented as frequencies and percentages. The influence of ALL genes, *BRCA1/2*, *CHEK2*, and *PALB2* on overall survival was analyzed using the Kaplan-Meier product limit method and Cox regression models. Kaplan-Meier curves and hazard ratios (HR) with 95% confidence interval (CI) adjusted for age at study entry, body mass index, hormone receptor status, HER2 status, grading, and therapy line are shown.

Figure 1: Patient selection and genotyping

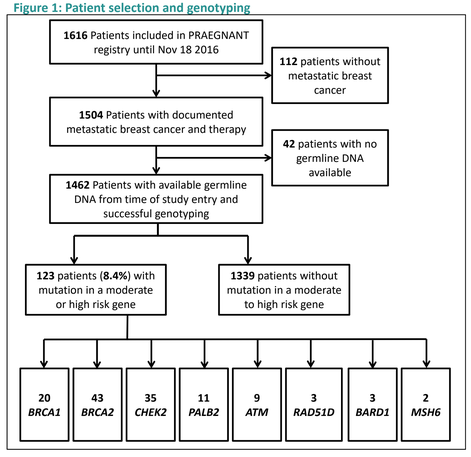


Figure 2: Mutation rates (in %) in subgroups according to molecular subtype

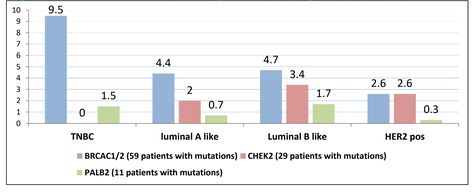
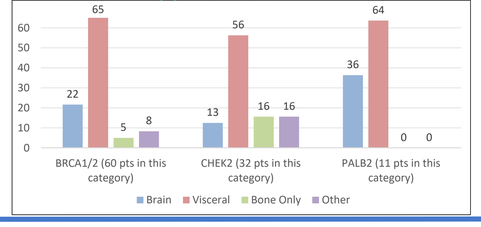


Figure 3: Distribution of metastasis location (in %) in subgroups of patients with a mutation in *BRCA1/2, CHEK2* or *PALB2*



RESULTS

A total of 1462 patients with metastastic breast cancer and clinical data were genotyped (Fig. 1). In this population 8.4% (n=123) of patients had a deleterious mutation in one of the analyzed genes. Most frequent mutations were seen in BRCA2, CHEK2, BRCA1, PALB2 and ATM.

Distribution of mutations according to patient and tumor characteristics is shown in Table 1. While the study population had an average age of 61.5 (±12.5) years, patients with a BRCA1/2 mutation were on average 51.8 (±11.8) years old. Patients with mutations in the other panel genes had an average age similar to the general study population.

The rate of mutations was highest in patients with triple negative breast cancer (TNBC) and luminal B like breast cancer. Mutations were detected in 12.4% (n=17) and 11.2% (n=26) of TNBC and luminal B like patients, respectively. Mutations were detected in 7.9% (n=47) and 6.0% (n=21) of patients with luminal A like or HER2 positive breast cancer, respectively (Table 1). Mutation rates according to molecular subtype for BRCA1/2, CHEK2 and PALB2 are shown in Figure 2.

With regard to therapy, the frequency of mutations was more pronounced in patients receiving first and second line therapy (8.4% and 10.7%), while mutation rates in patients receiving third line therapy or greater were 7.1% and 6.9%, respectively (Table 1).

Mutation rates (all genes) were highest in patients who developed brain or visceral metastases (9.9% and 9.1%) vs. those who had bone only or other metastases (5.2% and 7.1%) (Table 1), among patients developing metastases at a specific site (Figure 1). Patients with PALB2 mutations were more likely to develop brain metastases. Of all patients with a PALB2 mutation, 36.4% (n=4) developed brain metastases. None of the 11 PALB2 mutated patients developed a bone only metastatic pattern or a metastasis at another location (e.g. peritoneal, skin, …) (Figure 3).

The influence of mutations on overall survival was evaluated with four Cox regression models, one for each of the following predictors: Mutation in any of the analyzed genes, mutation in *BRCA1/2*, mutation in *CHEK2*, mutation in *PALB2*. Kaplan Meier Curves for these variables are shown in Figures 4-7.

Mutations in any of the genotyped genes had an influence on prognosis, with a HR of 1.48 (95%CI: 1.03-2.12). The other variables had similar effects, but these associations were not statistically significant possibly due to low sample sizes (Figures 4-7).

CONCLUSION

- Mutations in high and moderate risk breast cancer genes were frequent in metastatic breast cancer patients.
- The frequency was substantially higher than the 4% mutation frequency observed among unselected primary breast cancer patients, but is consistent with recent results from studies of metastatic prostate cancer patients.
- Patients with brain and visceral metastases had the highest BRCA mutation rates.
- Results suggested that *PALB2* mutations may be more frequent in patients with brain metastases.

ACKNOWLEDGEMENT

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Figure 4: Kaplan-Meier curves according to mutation status in any of the analyzed moderate to high risk genes

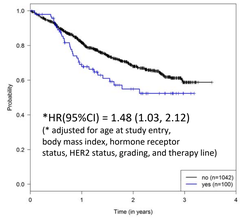

Figure 5: Kaplan-Meier curves according to BRCA1/2 mutation status

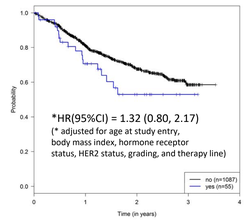

Figure 6: Kaplan-Meier curves according to CHEK2 mutation status

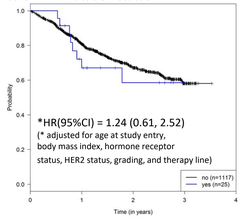

Figure 7: Kaplan-Meier curves according to PALB2 mutation status

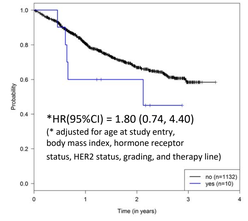

Table 1: Distribution of mutations according to patient and tumor characteristics

		Total	BRCA1 or BRCA2	Any other Gene	Any Gene	BRCA1	BRCA2	CHEK2	PALB2	ATM	RAD51D	BARD1	MSH6
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)		61.5 (12.5)	51.8 (11.8)	59.5 (11.4)	55.7 (12.1)	50.7 (12.0)	52.3 (11.8)	58.1 (10.3)	61.7 (13.2)	64.3 (12.6)	65.7 (13.8)	56.7 (12)	46 (1.4)
BMI		26.3 (5.6)	26.1 (5.1)	26.9 (6.6)	26.5 (5.9)	25.8 (4.2)	26.2 (5.5)	28.3 (7.3)	26.4 (7.4)	24 (4.0)	22.4 (0.4)	26.8 (1.8)	23.8 (NA)
Years since diagnosis		4.9 (6.3)	5.2 (6.3)	5.5 (6.5)	5.4 (6.4)	6.7 (8.2)	4.4 (5.2)	6.1 (6.9)	6 (6.0)	5.9 (8.0)	2.3 (2.1)	1.4 (1.4)	0 (NA)
		Total	BRCA1 or BRCA2	Any other Gene	Any Gene	BRCA1	BRCA2	CHEK2	PALB2	ATM	RAD51D	BARD1	MSH6
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Molecular Type	TNBC	137 (100)	13 (9.5)	4 (2.9)	17 (12.4)	9 (6.6)	4 (2.9)	0 (0.0)	2 (1.5)	1 (0.7)	0 (0.0)	1 (0.7)	0 (0.0)
	luminal A like	596 (100)	26 (4.4)	23 (3.9)	47 (7.9)	6 (1.0)	20 (3.4)	12 (2.0)	4 (0.7)	4 (0.7)	1 (0.2)	1 (0.2)	1 (0.2)
	luminal B like	232 (100)	11 (4.7)	16 (6.9)	26 (11.2)	2 (0.9)	9 (3.9)	8 (3.4)	4 (1.7)	2 (0.9)	1 (0.4)	1 (0.4)	0 (0.0)
	HER2 pos	352 (100)	9 (2.6)	12 (3.4)	21 (6.0)	1 (0.3)	8 (2.3)	9 (2.6)	1 (0.3)	1 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)
Grading	1	47 (100)	1 (2.1)	1 (2.1)	1 (2.1)	0 (0.0)	1 (2.1)	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	2	723 (100)	29 (4.0)	29 (4.0)	57 (7.9)	6 (0.8)	23 (3.2)	14 (1.9)	6 (0.8)	5 (0.7)	2 (0.3)	1 (0.1)	1 (0.1)
	3	483 (100)	29 (6.0)	22 (4.6)	50 (10.4)	12 (2.5)	17 (3.5)	11 (2.3)	5 (1.0)	3 (0.6)	1 (0.2)	2 (0.4)	0 (0.0)
Location of metastasis	brain	223 (100)	13 (5.8)	10 (4.5)	22 (9.9)	4 (1.8)	9 (4.0)	4 (1.8)	4 (1.8)	0 (0.0)	1 (0.4)	1 (0.4)	0 (0.0)
	visceral	805 (100)	39 (4.8)	34 (4.2)	73 (9.1)	13 (1.6)	26 (3.2)	18 (2.2)	7 (0.9)	5 (0.6)	1 (0.1)	1 (0.1)	2 (0.2)
	bone	193 (100)	3 (1.6)	8 (4.1)	10 (5.2)	1 (0.5)	2 (1.0)	5 (2.6)	0 (0.0)	3 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)
	other	184 (100)	5 (2.7)	8 (4.3)	13 (7.1)	1 (0.5)	4 (2.2)	5 (2.7)	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)
Therapy line at inclusion	1	770 (100)	34 (4.4)	33 (4.3)	65 (8.4)	11 (1.4)	23 (3.0)	20 (2.6)	5 (0.6)	3 (0.4)	1 (0.1)	2 (0.3)	2 (0.3)
	2	262 (100)	13 (5.0)	15 (5.7)	28 (10.7)	4 (1.5)	9 (3.4)	8 (3.1)	2 (0.8)	4 (1.5)	1 (0.4)	0 (0.0)	0 (0.0)
	3	182 (100)	5 (2.7)	8 (4.4)	13 (7.1)	2 (1.1)	3 (1.6)	1 (0.5)	4 (2.2)	2 (1.1)	0 (0.0)	1 (0.5)	0 (0.0)
	4+	248 (100)	11 (4.4)	7 (2.8)	17 (6.9)	3 (1.2)	8 (3.2)	6 (2.4)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)

Table 2: Distribution of mutations according to risk factors for breast cancer

		Total	BRCA1 or BRCA2	Any other Gene	Any Gene	BRCA1	BRCA2	CHEK2	PALB2	ATM	RAD51D	BARD1	MSH6
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Diagnosis before 46	no	1033 (100)	28 (2.7)	41 (4.0)	68 (6.6)	7 (0.7)	21 (2.0)	22 (2.1)	7 (0.7)	6 (0.6)	2 (0.2)	2 (0.2)	2 (0.2)
	yes	429 (100)	35 (8.2)	22 (5.1)	55 (12.8)	13 (3.0)	22 (5.1)	13 (3.0)	4 (0.9)	3 (0.7)	1 (0.2)	1 (0.2)	0 (0.0)
Bilateral breast cancer before age 51	no	1380 (100)	50 (3.6)	55 (4.0)	102 (7.4)	15 (1.1)	35 (2.5)	29 (2.1)	10 (0.7)	9 (0.7)	3 (0.2)	2 (0.1)	2 (0.1)
	yes	82 (100)	13 (15.9)	8 (9.8)	21 (25.6)	5 (6.1)	8 (9.8)	6 (7.3)	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	0 (0.0)
Diagnosis before 51 and one further 1st line relative with BC	no	1355 (100)	48 (3.5)	50 (3.7)	96 (7.1)	17 (1.3)	31 (2.3)	27 (2.0)	9 (0.7)	8 (0.6)	2 (0.1)	2 (0.1)	2 (0.1)
	yes	107 (100)	15 (14.0)	13 (12.1)	27 (25.2)	3 (2.8)	12 (11.2)	8 (7.5)	2 (1.9)	1 (0.9)	1 (0.9)	1 (0.9)	0 (0.0)
Diagnosis before 61 and TNBC	no	1374 (100)	53 (3.9)	60 (4.4)	110 (8.0)	14 (1.0)	39 (2.8)	35 (2.5)	9 (0.7)	9 (0.7)	3 (0.2)	2 (0.1)	2 (0.1)
	yes	88 (100)	10 (11.4)	3 (3.4)	13 (14.8)	6 (6.8)	4 (4.5)	0 (0.0)	2 (2.3)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)
Diagnosis after 51 and one 1st line relative with BC under 50	no	1434 (100)	59 (4.1)	62 (4.3)	118 (8.2)	19 (1.3)	40 (2.8)	34 (2.4)	11 (0.8)	9 (0.6)	3 (0.2)	3 (0.2)	2 (0.1)
	yes	28 (100)	4 (14.3)	1 (3.6)	5 (17.9)	1 (3.6)	3 (10.7)	1 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
More than 2 BC cases in this family	no	1430 (100)	61 (4.3)	56 (3.9)	114 (8.0)	20 (1.4)	41 (2.9)	30 (2.1)	9 (0.6)	9 (0.6)	3 (0.2)	3 (0.2)	2 (0.1)
	yes	32 (100)	2 (6.2)	7 (21.9)	9 (28.1)	0 (0.0)	2 (6.2)	5 (15.6)	2 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1st line relative with ovarian cancer	no	1425 (100)	55 (3.9)	62 (4.4)	114 (8.0)	16 (1.1)	39 (2.7)	34 (2.4)	11 (0.8)	9 (0.6)	3 (0.2)	3 (0.2)	2 (0.1)
	yes	37 (100)	8 (21.6)	1 (2.7)	9 (24.3)	4 (10.8)	4 (10.8)	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Male relative with BC	no	1443 (100)	61 (4.2)	63 (4.4)	121 (8.4)	20 (1.4)	41 (2.8)	35 (2.4)	11 (0.8)	9 (0.6)	3 (0.2)	3 (0.2)	2 (0.1)
	yes	19 (100)	2 (10.5)	0 (0.0)	2 (10.5)	0 (0.0)	2 (10.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Own history of ovarian cancer	no	1457 (100)	61 (4.2)	62 (4.3)	121 (8.3)	19 (1.3)	42 (2.9)	34 (2.3)	11 (0.8)	9 (0.6)	3 (0.2)	3 (0.2)	2 (0.1)
	yes	5 (100)	2 (40.0)	1 (20.0)	2 (40.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Any reason for predictive genetic testing	no	881 (100)	16 (1.8)	30 (3.4)	46 (5.2)	3 (0.3)	13 (1.5)	14 (1.6)	5 (0.6)	6 (0.7)	2 (0.2)	1 (0.1)	2 (0.2)
	yes	581 (100)	47 (8.1)	33 (5.7)	77 (13.3)	17 (2.9)	30 (5.2)	21 (3.6)	6 (1.0)	3 (0.5)	1 (0.2)	2 (0.3)	0 (0.0)

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