Original Article

Genetic Predisposition to Male Breast Cancer

(male breast cancer / hereditary cancer predisposition / germline genetic testing / NGS)

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Abstract. Male breast cancer (mBC) is a rare cancer diagnosis that constitutes less than 1 % of all breast cancer cases globally. Genetic factors play an important role in the mBC risk. Germline pathogenic variants (PVs) in cancer predisposition genes could be identified in about 15 % of cases. We performed germline genetic testing in 248 Czech mBC patients and 3,626 non-cancer male controls using next-generation sequencing by the CZECANCA panel (226 genes). We identified 46/248 (18.5 %) carriers of PVs in the established mBC predisposition genes, primarily in *BRCA2* (N = 34), less frequently in *BRCA1* (N = 7) and *PALB2* (N = 5). The presence of a PV in these genes significantly increased the mBC risk (OR 44.04; 5.82; and 8.26, respectively). Additionally, we identified 16 carriers of PVs in candidate mBC genes, but only PVs in *CHEK2* were significantly associated with increased mBC risk (OR = 4.98). The significance of 26 germline alterations in 23/192 additionally analysed genes remained uncertain. The carriers of PVs in *BRCA1* and *CHEK2* were significantly younger (55.8 and 52.6 years, respectively) than non-carriers (64.8 years), and all carriers of PVs in the established genes had more frequently grade G3 tumours and positive family cancer history. Our study underscores the critical role of *BRCA2* in mBC predisposition while also highlighting the potential contributions of additional genes that warrant further investigation. Moreover, it supports and justifies universal genetic testing for all mBC patients to generally improve early cancer detection and tailored treatment.

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Abbreviations: ACMG – American College of Medical Genetics, BAM – binary alignment map, CI – confidence interval, CNV – copy number variation, CZECANCA – CZEch CAncer paNel for Clinical Application, DCIS – ductal carcinoma *in situ,* ER – oestrogen receptor, ExAC – Exome Aggregation Consortium, fBC – female breast cancer, GATK – Genome Analysis Toolkit, gnomAD – The Genome Aggregation Database, HER2 – human epidermal growth factor receptor 2, HBOC – hereditary breast and ovarian cancer, IDC – invasive ductal carcinoma, IGV – integrative genomics viewer, ILC – invasive lobular carcinoma, mBC – male breast cancer, MLPA – multiplex ligation-dependent probe amplification, NCCN – National Comprehensive Cancer Network, NGS – next-generation sequencing, OC – ovarian cancer, OR – odds ratio, PaC – pancreatic cancer, PR – progesterone receptor, PrC – prostate cancer, PRS – polygenic risk score, PV – pathogenic variant, SAM – sequence alignment map, SC2 – SeqCap v.2, VCF – variant call format.

Introduction

Unlike female breast cancer (fBC), male breast cancer (mBC) is a rare oncological diagnosis. It accounts for < 1 % of all BC cases and 1 % of all cancers in men (Mukherjee et al., 2023). In 2022, 75 males were diagnosed with BC in the Czech Republic, more than twice as much as in 1990 (28 males). The annual mortality rate in the Czech Republic has been 20 deaths since 1990s (www.svod.cz). Due to its low prevalence, mBC is understudied and most screening and/or therapeutic recommendations are based on fBC studies and approaches.

The lifetime risk of BC in a general male population is about 0.1 %. The risk of mBC is increased by several non-genetic factors, such as obesity, alcohol consumption and high oestrogen levels (Khan and Tirona, 2021). The incidence of BC in the male population increases with age; the median age at diagnosis is 67 years, thus later than in females (Valentini et al., 2024). Genetic factors increase the mBC risk significantly. A positive family history and germline pathogenic variants (PVs) in cancer predisposition genes are the most important of them. Patients with Klinefelter syndrome (karyotype 47, XXY) have 20 × higher risk of mBC than average men (Swerdlow et al., 2005).

PVs in cancer predisposition genes have been present in 8–29 % of mBC cases (Table 1), with *BRCA2* PVs being by far the most frequent (4–40 % of mBC cases) and *BRCA1* PVs detected less frequently (0–5 % of mBC cases). The absolute lifetime risk of mBC rises to 5–10 % in *BRCA2* PV carriers and to 1–2 % in *BRCA1* PV carriers (Zheng and Leone, 2022). An increased risk of mBC (~ 1 %) is also described for carriers of PVs in other high-penetrance (*PALB2*) or moderate-penetrance (*ATM*, *CHEK2*) genes (Campos et al., 2021). All mBC patients are indicated for germline genetic testing according to the NCCN guidelines (www.nccn.org/guidelines) or the Czech national guidelines (Kleiblová et al., 2024b). Only a few studies have been published so far investigating the mBC genetic predisposition by panel NGS (targeting 3–585 genes; Table 1). Importantly, the list of analysed genes and frequency of identified PVs differs in various studies and populations, so the overall genetic landscape of mBC has not been comprehensively studied. Recently, the polygenic inheritance aggregated in polygenic risk score (PRS) has been shown to influence the mBC risk similarly to fBC (Maguire et al., 2021).

Regarding the clinical characteristics, mBC cases are mostly represented by invasive ductal carcinoma, less frequently by ductal carcinoma *in situ*. Other histological types are very rare. mBC is often grade 2, hormone receptor positive and HER2 negative. In comparison to fBC patients, mBC patients display worse prognosis with shorter overall survival (Pensabene et al., 2022).

Table 1. A list of published multi-gene panel NGS reports in mBC patients, including unselected, BRCA1/BRCA2-negative (*) or BRCA1/BRCA2/PALB2-negative (**) cases, respectively

Publication/population	# of patients	# of analysed genes	Most frequently altered genes	# of PVs; (%)
(Pritzlaff et al., 2017)/ United States	708[a]	7–49	*BRCA2* *BRCA1* *PALB2*	55 (8.1) 6 (0.9) 5 (0.8)
			All established genes	**66 (9.8)**
			CHEK2 *ATM*	16 (2.8) 6 (1.0)
(Fostira et al., 2018)/ Greece	102	94	*BRCA2* *BRCA1* *PALB2*	7 (6.9) 1 (1.0) 0
			All established genes	**8 (7.9)**
			ATM *CHEK2* *PMS2*	2 (2.0) 1 (1.0) 1 (1.0)
(Scarpitta et al., 2019)/ Italy	81	21	*BRCA2* *BRCA1* *PALB2*	18 (22.2) 0 0
			All established genes	**18 (22.2)**
			BRIP1 *MUTYH* *PMS2*	2 (2.5) 1 (1.2) 1 (1.2)
(Tedaldi et al., 2020)/ Italy	70	94	*BRCA2* *BRCA1* *PALB2*	6 (8.6) 3 (4.3) 1 (1.4)
			All established genes	**10 (14.3)**
			CHEK2 *ATM*	1 (1.4) 1 (1.4)
(Rolfes et al., 2022)/ Germany	614	23	*BRCA2* *BRCA1* *PALB2*	142 (23.1) 28 (4.6) 6 (1.0)
			All established genes	**176 (28.7)**
			CHEK2 *ATM*	8 (1.3) 4 (0.7)
(Evans et al., 2024)/ United Kingdom	204[a]	3–10	*BRCA2* *BRCA1* *PALB2*	51 (25.0) 5 (2.5) 0
			All established genes	**56 (27.5)**
			CHEK2	5 (2.5)
(Bucalo et al., 2023)*/ Italy	767	50	*PALB2* *ATM* *BLM* *FANCM* *CHEK2*	37 (4.8) 7 (0.9) 4 (0.5) 4 (0.5) 3 (0.4)
(Al Saati et al., 2023)**/ France	85	585	*CYP1B1* *ERCC2* *PALLD* *RECQL4* [b]genes with 1 PV	3 (3.5) 3 (3.5) 2 (2.4) 1 (1.2)

Notes: [a]not all men were tested by the same panel of genes (N = 512–677); [b]*CDKN2A, HOXA9, NUTM2A, PRCC, WRN, MRE11, BARD1, MUTYH, RAD51C, XPC*

The aim of our study was to analyse the germline genetic predisposition in a cohort of 248 Czech mBC patients and 3,626 male population-matched controls using NGS targeting 226 genes and to evaluate the clinicopathological characteristics in identified PV carriers.

Methods

Patients and controls

For this retrospective multicentric cohort study, we collected 248 mBC patients diagnosed at ten Czech health care centres (Supplementary Table S1). Of note,

all mBC cases are patients eligible for germline genetic testing reimbursed by the Czech national health insurance system. Patients were enrolled between 2000–2024 and were Caucasians of the Czech origin. Clinicopathological characteristics of all 248 patients are included in Table 2. The mean age at diagnosis in our mBC patients' cohort was 63.5 (range 30–89 years). Only 33 (14.4 %) cases were diagnosed at the age ≤ 50. The tumours were predominantly invasive ductal carcinomas (86.6 %), ER-positive (97.7 %), PR-positive (92.1 %), HER2-negative (87.3 %). Double primaries (bilateral BC, or second primary pancreatic (PaC) or prostate (PrC) cancer) were present in 8.9 % of mBC cases. A positive family cancer history considering first/second-degree relatives with ovarian (OC) or PaC (at any age) or BC/PrC (1 × at < 50 years or 2 × at any age) was present in 22.3 % of the patients.

Two control groups were considered. The "super-controls", consisting of 789 healthy individuals who were older than 60 years (92 males and 697 females) with a negative personal cancer history and any cancer in a first-degree relative, were used only for variant prioritization of NGS results. A group of 3,626 unselected population-matched male individuals was used for case-control analyses.

The study was approved by the Ethics Committees of the participating institutions. Written informed consent for the research analysis was obtained from all participants. Clinicopathological information was collected during genetic counselling or retrieved from the patients' records.

NGS sequencing

One hundred ng of genomic DNA extracted from peripheral blood and collected at the time of enrolment in each respective centre was used to prepare the NGS library (KAPA HyperPlus Kit; Roche, Basel, Switzerland) according to the manufacturer's instructions, as we described previously (Soukupova et al., 2016; 2018). Target regions were enriched by hybridization with custom-designed panel CZECANCA (CZEch CAncer paNel for Clinical Application), targeting 226 genes (entire coding and adjacent intron regions) known or candidate for cancer predisposition (www.czecanca.cz/en), in 190 patients and all controls, or panel SC2 targeting 54 genes (used by centre #6), out of which 51 overlapped with the CZECANCA panel (in 58 mBC patients; Supplementary Table S2). Prepared and quantified libraries were sequenced in Illumina NGS instrument NextSeq500 using chemistry NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles). Sequencing data were analysed using an in-house bioinformatic pipeline with minor modifications described previously (Soukupova et al., 2018; Kral et al., 2023). Briefly, germline variants were aligned to the human genome hg19. SAM files were generated from FASTQ using NovoAlign and transformed into BAM by Picard tools. GATK was used to prepare VCF, annotated by SnpEff. Identification of medium size in-

Table 2. Clinicopathological characteristics of the patients' group

Subgroup	Number of patients (%, from known)
Age at dg (years); median (range)	63.5 (30–89 years)
< 40 years	14 (6.1)
41–50 years	19 (8.3)
51–60 years	51 (22.3)
61–70 years	71 (31.0)
71–80 years	63 (27.5)
> 80 years	11 (4.8)
NA	19 (7.7)
Personal cancer history	
mBC unilateral	204 (82.3)
mBC bilateral	6 (2.4)
mBC + PaC/PrC	16 (6.5)
mBC + other cancer	22 (8.9)
Family cancer history	
HBOC negative	162 (74.7)
HBOC positive	55 (22.3)
NA	31 (12.5)
mBC histology	
IDC	161 (86.6)
DCIS	9 (4.8)
ILC	5 (2.7)
other	11 (5.9)
NA	62 (14.9)
Grade	
1	24 (14.7)
2	88 (54.0)
3	51 (31.3)
NA	85 (34.3)
Classification	
Basal	4 (3.2)
Luminal A	29 (23.4)
Luminal B	91 (73.4)
NA	124 (49.2)
Stage	
In situ	7 (5.5)
I	37 (28.9)
II	51 (39.8)
III	29 (22.7)
IV	4 (3.1)
NA	120 (48.4)
ER status	
ER^+	173 (97.7)
ER^-	4 (2.3)
NA	71 (28.6)
PR status	
PR^+	163 (92.1)
PR^-	14 (7.9)
NA	71 (28.6)
HER2 status	
$HER2^+$	20 (12.7)
$HER2^-$	137 (87.3)
NA	91 (36.7)

Note: BC – breast cancer; OC – ovarian cancer; PaC – pancreatic cancer; PrC – prostate cancer; DCIS – ductal carcinoma *in situ*; IDC – invasive ductal carcinoma; ILC – invasive lobular carcinoma; NA – not available.

dels was performed by Pindel and copy number variations (CNVs) were detected using a CNV kit.

Variant prioritization

All 226 genes were divided according to their association with mBC. Only *BRCA1*, *BRCA2* and *PALB2* were considered "established" mBC predisposition genes. Further 31 "candidate" mBC predisposition genes were selected as they had been analysed in fBC patients in previous large-scale studies (Dorling et al., 2021; Hu et al., 2021) and are listed in Table 3. The remaining 192 genes were referred to as "other" genes.

Table 3. List of fBC predisposition genes according to two previous large-scale studies of fBC. Established mBC genes are highlighted in ***bold*** *letters.*

mBC predisposition genes	**(Hu et al., 2021) (N = 26)**	**(Dorling et al., 2021) (N=31)**
Established (N = 3)	***BRCA1***	***BRCA1***
	BRCA2	***BRCA2***
	PALB2	***PALB2***
Candidate (N = 31)	*ATM*	*ATM*
	–	*BABAM1*
	BARD1	*BARD1*
	BLM	–
	BRIP1	*BRIP1*
	CDH1	*CDH1*
	–	*EPCAM*
	CDKN2A	–
	–	*FAM175A*
	FANCC	*FANCC*
	FANCM	*FANCM*
	CHEK2	*CHEK2*
	–	*MEN1*
	MLH1	*MLH1*
	MRE11A	*MRE11A*
	MSH2	*MSH2*
	MSH6	*MSH6*
	–	*MUTYH*
	NBN	*NBN*
	NF1	*NF1*
	–	*PIK3CG*
	–	*PMS2*
	PTEN	*PTEN*
	RAD50	*RAD50*
	RAD51C	*RAD51C*
	RAD51D	*RAD51D*
	RECQL	*RECQL*
	SLX4	–
	–	*STK11*
	TP53	*TP53*
	XRCC2	*XRCC2*

"–" indicates candidate genes not involved in the respective study

Identified variants were classified according to their pathogenicity using a 5-tier system. Only likely/pathogenic variants (class 4/5; referred to as "PVs") were considered for further analysis.

Variant filtration excluded all variants with sequence quality less than 150, those localized in repetitive or non-coding sequences and sequencing errors. Variants with minor allele frequency (MAF) > 0.4 % in the "super-control" group were excluded as well as those with MAF > 0.4 % in one or more online accessible databases (gnomAD, 1000 Genomes Project, ExAC databases). Variants were classified according to the national consensus based on published ACMG recommendations (Richards et al., 2015; Janatová et al., 2023). Variants classified as benign/likely benign in the ClinVar database and synonymous or in-frame indels variants were excluded unless classified as class 4/5. Frame-shift, stop-gain and splice-site (± 1/2) were considered PVs. CNVs were considered pathogenic in case of any deletion or duplication not involving first or last exons. Missense variants were considered class 4/5 when classified as such in ClinVar or when confirmed pathogenic by well-established functional analysis (Stolarova et al., 2023).

Pathogenic variants were confirmed by Sanger sequencing or Multiplex Ligation-dependent Probe Amplification (MLPA) analysis or visually inspected in IGV. Variants located within exon-intron junctions were analysed at the RNA level to confirm their impact on aberrant splicing using RNA-based targeted sequencing, as we described recently (Kleiblová et al., 2024a; Zemankova et al., 2024).

Statistical analysis

The odds ratio (OR) and 95% confidence interval (95% CI) were calculated by the Fisher's exact test. Unselected population-matched male control individuals were used. The Mann-Whitney test was used to calculate differences in the mean age between subgroups. Statistical analyses were performed in R v.4.2.0. Statistical tests were two-sided and the P value of < 0.05 was considered statistically significant. P values indicated by asterisks in Figures 2 and 3 are: $^{*}< 0.05$; $^{**}< 0.01$.

Results

Our NGS analysis targeting 226 cancer predisposition genes identified 91 PVs in 80/248 (32.3 %) Czech mBC patients.

PVs in three established mBC predisposition genes (Table 4) were found in 46/248 (18.5 %) patients, most frequently in *BRCA2* (N = 34; 13.7 %), followed by *BRCA1* (N = 7; 2.8 %) and *PALB2* (N = 5; 2.0 %). One *BRCA2* PV carrier was also diagnosed with Klinefelter syndrome (KS).

PVs in eight of 31 candidate mBC predisposition genes (Table 4) were found in 19/248 (7.7 %) patients. Three *CHEK2* PV carriers also had a PV in *BRCA2*. For further calculations, these three patients were included

Table 4. Risks associated with PVs in mBC predisposition genes. Statistically significant OR values are highlighted in ***bold****.*

Gene	Carriers in 248 patients; N (%)	Carriers in 3,626 controls; N (%)	Odds ratio (95% CI)	P value	P_{adj} value
BRCA1	**7 (2.8)**	**18 (0.5)**	**5.82 (2.03–14.77)**	**0.0007**	**0.009**
BRCA2	**34 (13.7)**	**13 (0.4)**	**44.04 (22.25–92.33)**	**1.9×10^{-31}**	**2.7×10^{-30}**
PALB2	**5 (2.0)**	**9 (0.2)**	**8.26 (2.16–27.71)**	**0.001**	**0.015**
All established genes	**46 (18.5)**	**40 (1.1)**			
ATM	2 (0.8)	23 (0.6)	1.27 (0.14–5.20)	0.67	1
BLM	1 (0.4)	20 (0.6)	0.73 (0.02–4.60)	1	1
BRIP1	1 (0.4)	6 (0.2)	2.44 (0.05–20.25)	0.37	1
CHEK2[a]	**10 (4.0)**	**21 (0.6)**	**7.20 (2.99–16.20)**	**0.00001**	**0.0001**
CHEK2[b]	**7 (2.8)**	**21 (0.6)**	**4.98 (1.77–12.32)**	**0.0015**	**0.021**
FANCM	2 (0.8)	19 (0.5)	1.54 (0.17–6.45)	0.39	1
NBN	1 (0.4)	17 (0.5)	0.86 (0.02–5.53)	1	1
RAD50	1 (0.4)	10 (0.3)	1.46 (0.03–10.36)	0.52	1
SLX4	1 (0.4)	4 (0.1)	3.66 (0.07–37.17)	0.28	1
All candidate genes[b]	**16 (6.5)**	**120 (3.3)**			

Note: Risk for *CHEK2* is calculated including (a) and excluding (b) *BRCA2* + *CHEK2* double PV carriers.

in the group of PV carriers in the established mBC predisposition genes. Thus, PVs in candidate mBC genes exclusively were found in 16/248 (6.5 %) patients, most frequently in *CHEK2* (N = 7; 2.8 %), followed by *ATM* and *FANCM* (two carriers each; 0.8 %) and *BLM*, *BRIP1*, *NBN*, *RAD50* and *SLX4* (one carrier each; 0.4 %) (Fig. 1). All identified PVs together with clinical characteristics of the carriers are listed in Supplementary Table S3.

Additionally, we identified PVs in 23 out of 192 "other" genes (listed in Supplementary Table S4) in 18/248 (7.3 %) mBC patients (carriers of PVs in established or candidate mBC predisposition genes were not considered). Each gene mostly contained one PV, except for *RECQL4* (N = 3; 1.2 %) and *CLSPN* (N = 2; 0.8 %).

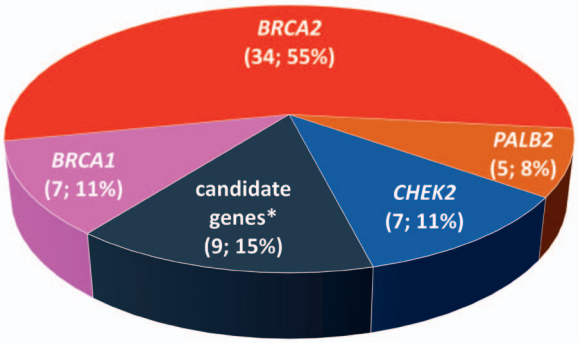

Fig. 1. Proportion of PVs in established (shades of red) and candidate (shades of blue) mBC predisposition genes in the cohort of 248 mBC patients. * # of PVs in candidate genes besides *CHEK2*: *ATM* (N = 2), *BLM* (N = 1), *BRIP1* (N = 1), *FANCM* (N = 2), *NBN* (N = 1), *RAD50* (N = 1), *SLX4* (N = 1).

Only two genes out of 23 "other genes" with PVs were also involved in the SC2 panel.

Next, we compared the frequencies of PVs in mBC predisposition genes between mBC patients and unselected population-matched male controls to calculate the risks associated with individual PVs (Table 4). All three established mBC predisposition genes were associated with statistically significantly increased risk (*BRCA2*: OR = 44.04; *PALB2*: OR = 8.26; *BRCA1*: OR = 5.82). Of the group of candidate mBC predisposition genes, only *CHEK2* PVs were significantly associated with increased mBC risk (both when the three *BRCA2* + *CHEK2* double PV carriers were included as well as excluded; OR = 7.20 and OR = 4.98, respectively).

In the group of 192 "other" genes, the mBC risk was significantly increased for *CLSPN* (OR = 14.82, 95% CI: 1.07–205.01, P = 0.022) and *RECQL4* (OR = 4.96, 95% CI: 0.86–20.03, P = 0.036) only without multiple testing correction (Supplementary Table S4). Risk calculations for the remaining "other" genes were not performed due to the small number of PV carriers.

We evaluated the clinical characteristics of PV carriers and compared the differences among the mean age at diagnosis of mBC in PV carriers in established, candidate and other mBC predisposition genes and in non-carriers. The mean age at the diagnosis of mBC was significantly lower for the carriers of PVs in *BRCA1* (mean 55.8 years) when compared to the non-carriers (mean 64.8 years; Fig. 2). The lower mean age at diagnosis was also observed for carriers of PV in any candidate gene (56.2 years), but this finding was likely mostly driven by the significantly lower mean age at diagnosis of six *CHEK2* PV carriers (52.6 years) who developed mBC at 35.5, 43.1, 44, 55.4, 66.4, and 71 years, respectively (the clinical data were unavailable

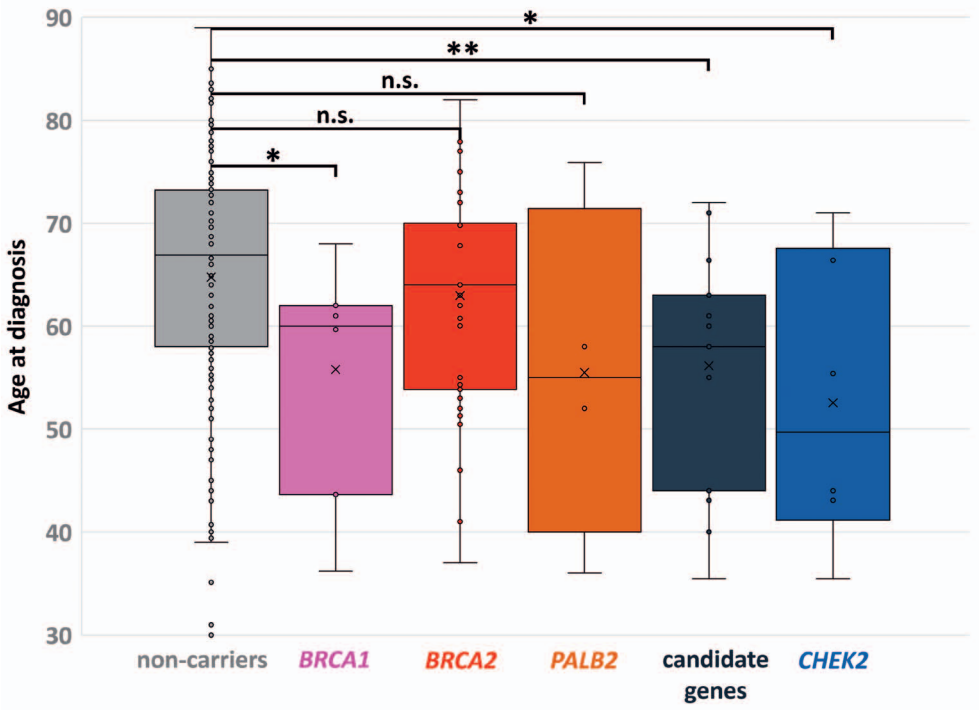

Fig. 2. Comparison of the mean age at mBC diagnosis in carriers of PVs in individual established mBC genes, candidate genes, *CHEK2* separately and non-carriers (including carriers of PVs in other genes); n.s. – not significant.

for one *CHEK2* PV carrier). Interestingly, the age at diagnosis was higher in the three *BRCA2* + *CHEK2* double PV carriers developing mBC at 55, 72 and 77.9 years (mean 68.3 years).

Multiple primary cancer diagnoses were observed in 7/46 (15.2 %) carriers of PVs in established mBC predisposition genes (all of them carried a *BRCA2* PV). Two patients were diagnosed with bilateral BC (one of them also with pancreatic cancer), three patients with prostate cancer and two patients with pancreatic cancer. One *CHEK2* PV carrier had a concurrent diagnosis of prostate cancer (1/16; 6.3 %). Among the non-carriers, 14/186 patients (7.5 %) were diagnosed with a second primary cancer, four patients with bilateral BC and 10 patients were diagnosed with mBC and prostate cancer (2 × at the same age, 5 × prostate cancer first, and 2 × mBC first; in one patient the age of prostate cancer onset was not available). Although the proportion of patients with a positive personal history of cancer was higher among the carriers of PV in three established mBC predisposition genes in comparison to non-carriers (15.2 % vs 7.5 %), the difference was not statistically significant (P = 0.15) (Fig. 3A).

A positive family cancer history was reported in 17/42 (40.5 %) carriers (where the family history was known) of PVs in established mBC genes, in 3/13 (23.1 %) carriers of PVs in candidate genes and in 35/162 (21.6 %) non-carriers. The difference was statistically significant only for carriers of PVs in the three established mBC predisposition genes in comparison to non-carriers (P = 0.017) (Fig. 3B).

Regarding the pathological characteristics, carriers of PVs in the established mBC genes have 3 × higher risk to develop grade 3 (G3) tumour than non-carriers (OR = 3.5, 95% CI: 1.4–8.81, P = 0.004) (Fig. 3C). No statistically significant differences were identified for other characteristics, including the histological tumour type or receptor status.

Discussion

Our multicentric study revealed 62 carriers of PVs in established and candidate mBC predisposition genes (25.0 %) among 248 unselected Czech mBC patients. Our study confirmed the dominant role of *BRCA2* in mBC genetic predisposition. Carriers of *BRCA2* PV represented 13.7 % of all mBC patients, 4 × more than carriers of PVs in *BRCA1*. The *BRCA2*-attributed cumulative lifetime mBC risk (OR = 44.04) reached the 5 % threshold (compared to 0.1 % reported for the general male population), justifying a specific clinical management (Valentini et al., 2024). Previous studies reported the presence of *BRCA2* PVs in 7–25 % of mBC patients (Table 1) (Pritzlaff et al., 2017; Fostira et al., 2018; Tedaldi et al., 2020; Rolfes et al., 2022; Evans et al., 2024). This range may reflect different population frequencies of *BRCA2* PVs in various populations. However, our study clearly documented a much stronger effect of *BRCA2* PVs over *BRCA1* PVs on the mBC risk, while the population frequency of *BRCA1* PVs dominates in the Czech population over that in *BRCA2* (as seen in the controls – 0.50 % vs. 0.36 % (Table 4)

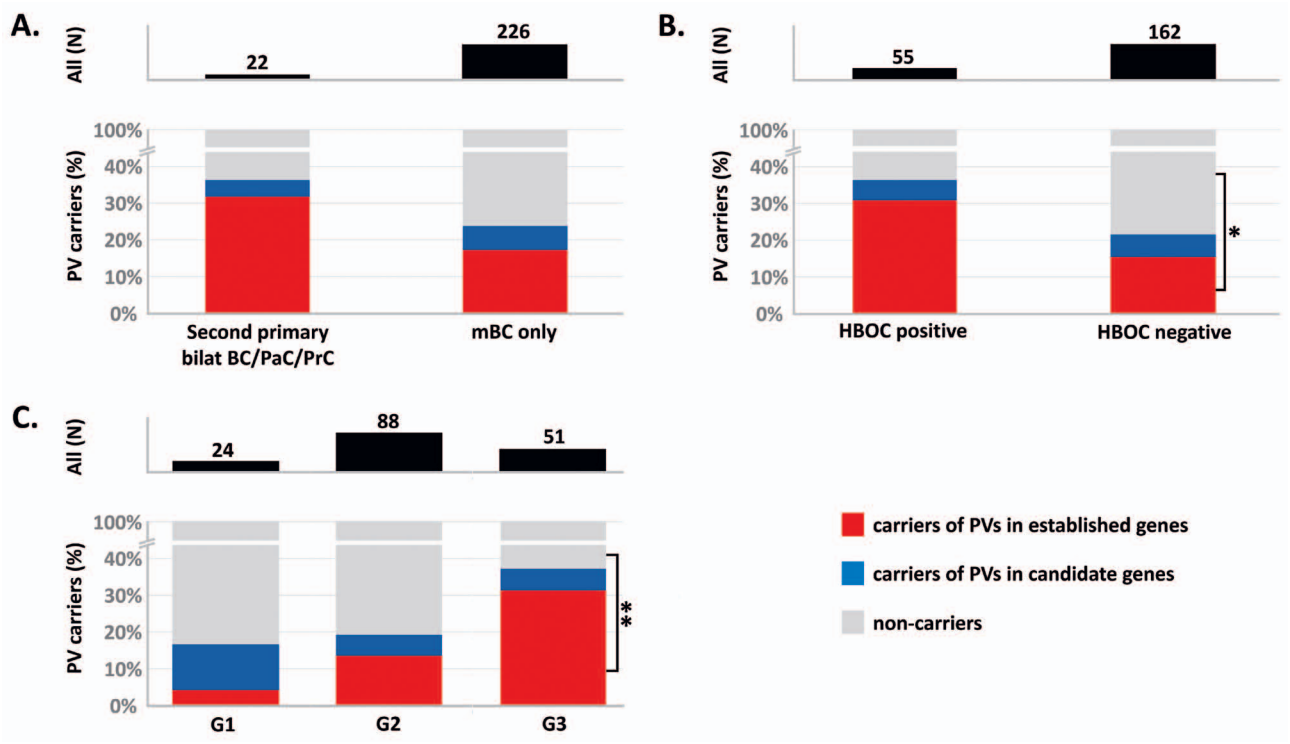

Fig. 3. Relative frequency of PV carriers according to **(A)** second primary, **(B)** family HBOC cancer history, and **(C)** tumour grading.

and previous studies in fBC patients (Pohlreich et al., 2005; Machackova et al., 2019)).

The frequency of PVs in two other established mBC predisposition genes, *BRCA1* and *PALB2*, was lower with correspondingly lower mBC risks (comparable with that found in previous reports). The prevalence of PVs in *BRCA1* in unselected mBC patients has been previously reported between 0–4 % (Rizzolo et al., 2013) and the cumulative mBC risk for male carriers about 0.4 % (4 × higher compared with the population mBC risk; (Li et al., 2022)). The risk of mBC associated with *PALB2* PVs was previously described as RR = 7.34 by a segregation analysis in families (Yang et al., 2020).

ATM and *CHEK2* are other fBC predisposition genes frequently associated with mBC in NGS studies (Table 1). Our results confirm a statistically significantly moderately increased risk only for *CHEK2* PV carriers (OR = 4.98). This agrees with our previous study in a much smaller set of 48 mBC patients (Kleiblova et al., 2019). However, the role of *CHEK2* in predisposing to mBC has been described inconsistently. Its PV frequencies in mBC patients and associated risks differ between various studies, from no mutation to the frequency of 4.1 % and OR = 2.4 (Pritzlaff et al., 2017; Rolfes et al., 2022; Al Saati et al., 2023; Bucalo et al., 2023). The prevalence of *CHEK2* PVs varies among different populations, which leads to inconsistent results of OR calculations in studies using population-matched controls and publicly available control datasets (Rump et al., 2016). For example, the geographical differences in overall *CHEK2* PV frequency are typically described as decreasing from the north to the south European countries (Kleibl et al., 2005). Moreover, the frequencies of *CHEK2* PVs are significantly lower in Asian populations than in populations of European ancestry (Stolarova et al., 2020). We did not find any mBC association with PVs in *ATM*. International studies with larger numbers of analysed patients are needed to conclusively estimate the role of *ATM* in mBC predisposition. Additionally, we identified PVs also in other candidate genes (including *BLM*, *BRIP1*, *FANCM*, *NBN*, *RAD50* and *SLX4*). While PVs in some of them have a known role in cancer predisposition, their role in mBC development needs to be confirmed in subsequent studies (Kluźniak et al., 2019; Stastna et al., 2024).

The overall high frequency of PVs in the established and candidate mBC predisposition genes (25.0 %) in mBC patients justifies the national and international recommendations for germline genetic testing in all mBC patients regardless of the age, personal/family cancer history, or tumour phenotype (Campos et al., 2021; Kleiblová et al., 2024b). A similar frequency of PV carriers in *BRCA2*, *BRCA1*, *CHEK2* and other candidate genes (22.4 %) was recently reported in 116 Czech mBC patients analysed by germline genetic testing (Bielcikova et al., 2024).

Individual studies of mBC patients using panel NGS vary in the number of analysed genes as well as in the number of enrolled patients (Table 1); however, no additional gene has been associated with increased mBC

risk convincingly. In the group of "other" genes, more than one PV was identified only in *RECQL4* and *CLSPN*, with a marginal statistical significance. PVs in *RECQL4* were previously identified in one mBC patient (Al Saati et al., 2023). Bi-allelic germline mutations in *RECQL4* cause the type II Rothmund-Thomson syndrome (RTS; OMIM #268400), characterized by a premature ageing phenotype and cancer predisposition (Martins et al., 2023). The *CLSPN* gene codes for claspin, which has a functional significance in the DNA damage response and replication; however, germline PVs in *CLSPN* have not been associated with cancer to date (Erkko et al., 2008). Additionally, we detected one PV in *ERCC2* (1/248; 0.4 %). PVs in *ERCC2* were also described in three mBC patients (3/85; 3.5 %) by Al Saati et al. (2023). PVs in *ERCC2* were previously identified in fBC in the Czech population, but without statistical significance when the frequency was compared to population-matched controls (1.4 % and 1.1 %, respectively) (Rump et al., 2016). Bi-allelic PVs in *ERCC2* are associated with *Xeroderma pigmentosum* type D (XPD, OMIM #278730), characterized by skin photosensitivity and early development of skin tumours (DiGiovanna and Kraemer, 2012). However, the possible role of these genes in mBC predisposition would have to be further confirmed.

Identification of cancer-associated PVs in mBC patients is of high importance for the patients as well as for their relatives considering the clinical implications. Healthy carriers of PVs can be referred for a surveillance according to gene- and gender-specific protocols and guidelines (Tedaldi et al., 2020; Kleiblová et al., 2024b). Moreover, the presence of PVs in *BRCA1/2* genes (and other genes involved in DNA repair via homologous recombination) can direct targeted therapy (platinum derivatives, PARP inhibitors).

We have shown that mBC carriers of PVs in *BRCA1* had statistically significantly lower age of mBC onset than non-carriers. However, this finding is not consistent with other studies showing that the age at the disease onset in carriers of PVs in *BRCA1/2* did not differ from that in con-carriers (Pritzlaff et al., 2017; Fostira et al., 2018; Tedaldi et al., 2020; Rolfes et al., 2022; Evans et al., 2024). Interestingly, the six carriers of a *CHEK2* PV (with available clinical data) in our study had a significantly lower mean age at diagnosis (52.6 years) than the non-carriers and the *BRCA2* PV carriers (64.8 and 63.0, respectively). This is in line with the findings by Evans et al. (2024) and Pritzlaff et al. (2017), who also detected earlier age at the BC onset in male carriers of a *CHEK2* PV. Recently, we described lower age at diagnosis also for ovarian cancer patients carrying a *CHEK2* PV (Horackova et al., 2024). However, the three carriers of concurrent *BRCA2* and *CHEK2* PVs in our study displayed higher age of diagnosis (55, 72 and 77.9 years), indicating that the tumour development was probably driven by a *BRCA2* PV associated with later onset. Due to the overall small number of *CHEK2* PV carriers, this finding needs to be evaluated in larger studies.

Carriers of PVs in established mBC predisposition genes were more likely to have a positive personal and family cancer history (the latter was statistically significant in our study). This agrees with previous studies (Pritzlaff et al., 2017; Rolfes et al., 2022) and corresponds to the autosomal dominant inheritance of cancer predisposition in the affected families. Interestingly, 10 mBC patients with no detected PVs were also diagnosed with prostate cancer. This could indicate the existence of another not yet known cancer predisposition gene(s) or possible polygenic background common for both diagnoses (Hassanin et al., 2022). Environmental and behavioural factors may also play an important role considering mBC as a multifactorial disease, similarly as other tumours.

We did not find any striking differences in the clinicopathological characteristics of tumours in PV carriers and non-carriers. Carriers and non-carriers displayed the same characteristics, with predominant ER-positive, PR-positive and HER2-negative tumour types, as in previous studies (Rolfes et al., 2022; Bielcikova et al., 2024; Evans et al., 2024). The only exception was a higher grade in carriers of PVs in established mBC genes. Clinicopathological characteristics have not been reported in previous studies of mBC genetic predisposition; therefore, future studies are necessary to confirm our observations. Our data also suggest that, in contrast to fBC, while *BRCA1* PV carriers mostly develop ER/PR-negative tumours (Guzmán-Arocho et al., 2022), *BRCA1*-positive mBC patients predominantly develop ER/PR-positive tumours. Whether this phenomenon may have a similar negative impact on prognosis in *BRCA1*-positive mBC patients as in *BRCA1*-positive ER-positive fBC patients remains to be established (Vocka et al., 2019). However, this difference may explain the overall poorer survival of *BRCA1/2*-positive mBC patients in comparison to wild-type *BRCA1/2* mBC patients (Gargiulo et al., 2016).

Our study is limited by its retrospective case-control design, which does not allow evaluation of outcomes, interventions, or absolute risk calculations. Also, the relatively small size of the patient cohort may cause a bias for calculations of associated cancer risks, especially for genes with very low PV population frequencies.

Conclusion

In conclusion, our results contribute to the understanding of the hereditary susceptibility to mBC in the Czech population. We confirmed the role of established mBC predisposition genes. However, our and previously published data also suggest that the *CHEK2* gene should be considered as an established mBC predisposition gene. The role of other known female BC predisposition genes is still unclear, and the comparison of the genetic landscape between female and male BC needs to be fully investigated. However, the results support the benefit of multi-gene panel testing in all mBC patients. The association between clinicopathological character-

istics and PV status in mBC patients is important for more accurate setting of clinical management and care.

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Conflict of interest

The authors declare no conflict of interest.

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