Spectrum of double heterozygosity in individuals diagnosed with hereditary breast and ovarian cancer

Natalie Herold, Christoph Engel, Dorothee Speiser, Tim Ripperger, Andrea Gehrig, Nadia Harbeck, Norbert Arnold, Nicola Dikow, Susanne Ledig, Elena Leinert, Matthias Rath, Jan Hauke, Susanne Morlot, Nina Ditsch, Julia Ritter, Britta Blümcke, Jörg Schröder, Anke Waha, Anna Hester, Monika Maringa, Bernadette A. S. Jäger, Rita Schmutzler, Pauline Wimberger, Barbara Wappenschmidt, Bjoern Sander, Christine Solbach, Bernhard H. F. Weber, Tobias Blaum, Christopher Schroeder, Alexander E. Volk, Bahriye Aktas, Marion Kiechle, Cornelia Meisel, Tanja Fehm, Eric Hahnen, Bernd Auber, Kerstin Rhiem, Monika M. Golas

Angaben zur Veröffentlichung / Publication details:

Herold, Natalie, Christoph Engel, Dorothee Speiser, Tim Ripperger, Andrea Gehrig, Nadia Harbeck, Norbert Arnold, et al. 2026. "Spectrum of double heterozygosity in individuals diagnosed with hereditary breast and ovarian cancer." *European Journal of Cancer* 244: 116874. https://doi.org/10.1016/j.ejca.2026.116874.

European Journal of Cancer 244 (2026) 116874

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Original Research

Spectrum of double heterozygosity in individuals diagnosed with hereditary breast and ovarian cancer

Natalie Herold [a], Christoph Engel [b], Dorothee Speiser [c], Tim Ripperger [d], Andrea Gehrig [e], Nadia Harbeck [f], Norbert Arnold [g], Nicola Dikow [h], Susanne Ledig [i], Elena Leinert [j], Matthias Rath [k,l], Jan Hauke [a], Susanne Morlot [d,l], Nina Ditsch [m], Julia Ritter [c,n], Britta Blümcke [a], Jörg Schröder [e], Anke Waha [a], Anna Hester [f], Monika Maringa [a], Bernadette A.S. Jäger [o,p], Rita Schmutzler [a], Pauline Wimberger [q], Barbara Wappenschmidt [a], Bjoern Sander [r], Christine Solbach [s], Bernhard H.F. Weber [t,u], Tobias Blaum [v], Christopher Schroeder [w], Alexander E. Volk [a,x], Bahriye Aktas [y], Marion Kiechle [z], Cornelia Meisel [q], Tanja Fehm [o,p], Eric Hahnen [a], Bernd Auber [d], Kerstin Rhiem [a], Monika M. Golas [d,aa,ab,*]

[a] Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology, Medical Faculty, University Hospital Cologne, Cologne, Germany
[b] Institute for Medical Informatics, Statistics and Epidemiology, Leipzig University, Leipzig, Germany
[c] Hereditary Breast and Ovarian Cancer Center Charité, Department of Gynecology with Breast Center, Charité University Medicine Berlin, Berlin, Germany
[d] Department of Human Genetics, Hannover Medical School, Hannover, Germany
[e] Institute of Human Genetics, Julius-Maximilians-Universität Würzburg, Würzburg, Germany
[f] Department of Gynecology and Obstetrics, Breast Center, LMU University Hospital, Munich, Germany
[g] Department of Gynecology and Obstetrics, Institute of Clinical Molecular Biology, University Hospital of Schleswig-Holstein, Christian-Albrechts-University Kiel, Kiel, Germany
[h] Institute of Human Genetics, Heidelberg University, Heidelberg, Germany
[i] Department of Medical Genetics, University Hospital Münster, Münster, Germany
[j] Department of Gynecology and Obstetrics, University Hospital Ulm, Ulm, Germany
[k] Department of Human Genetics, University Medicine Greifswald and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany
[l] Present address: Institute for Molecular Medicine, MSH Medical School Hamburg, Hamburg, Germany
[m] Gynaecology, Obstetrics and Senology, Faculty of Medicine, University of Augsburg, and BZKF (Bavarian Center for Cancer Research), Germany
[n] Labor Berlin – Charité Vivantes GmbH, Berlin, Germany
[o] Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany
[p] Center for integrated Oncology (CIO) ABCD, Düsseldorf, Germany
[q] Department of Gynecology and Obstetrics, Technische Universität Dresden, and NCT Dresden, Dresden, Germany
[r] Institute of Pathology, Hannover Medical School, Hannover, Germany
[s] Center for Hereditary Breast and Ovarian Cancer, University Hospital Frankfurt, Frankfurt, Germany
[t] Institute of Human Genetics, University of Regensburg, Regensburg, Germany
[u] Institute of Clinical Human Genetics, University Hospital Regensburg, Regensburg, Germany
[v] Department of Gynecology and Obstetrics, University Medicine Göttingen, Göttingen, Germany
[w] Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany
[x] Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[y] Department of Gynecology, University of Leipzig Medical Center, Leipzig, Germany
[z] Department of Gynecology and Center for Hereditary Breast and Ovarian Cancer, TUM University Hospital, Technical University Munich, Munich, Germany
[aa] Institute of Human Genetics, University Hospital Augsburg and Faculty of Medicine, University of Augsburg, Augsburg, Germany
[ab] Comprehensive Cancer Center Augsburg and Bavarian Cancer Research Center (BZKF), University Hospital Augsburg, Augsburg, Germany

* Correspondence to: Institute of Human Genetics, University Hospital Augsburg, and Faculty of Medicine, University of Augsburg, Stenglinstr. 2, Augsburg D-89156, Germany.
E-mail address: monika.golas@med.uni-augsburg.de (M.M. Golas).
[1] Present address: MVZ Amedes Genetics, Hannover, Germany

https://doi.org/10.1016/j.ejca.2026.116874
Received 24 April 2026; Accepted 11 May 2026
Available online 11 June 2026

ARTICLE INFO

Key words:
Multiple heterozygosity
MINAS
Hereditary breast and ovarian cancer
Severity score
Germline
Phenotype-genotype

ABSTRACT

Background: (Likely) pathogenic variants (pVs) in hereditary breast and/or ovarian cancer (HBOC) genes increase cancer risk, but the clinical implications of multiple pVs remain insufficiently understood.
Methods: Using data from the prospective nationwide German Consortium for HBOC registry, we conducted a case-control study of genotype-phenotype associations, matching female carriers to compare cancer risk and disease severity between multiple and single heterozygous (SH) pV carriers.
Results: Among 26,983 carriers of single or multiple HBOC-associated pVs, 409 individuals had multiple heterozygosities, including 400 double heterozygous (DH) carriers. In a subcohort tested for 13 core genes, 98/3407 (2.9%) individuals had multiple HBOC-associated pVs. Breast cancers (BCs) in DH women with pVs in *BRCA1* and another gene were predominantly triple-negative, even when the second, non-*BRCA1* pV was typically associated with hormone receptor (HR)-positive BC. By contrast, *BRCA1/CHEK2* DH carriers showed more HR-positive BCs than *BRCA1* SH women. The median age at first BC was similar in female *ATM/CHEK2* DH carriers and *BRCA1* SH carriers (41.5 [IQR 14.0] vs. 41.3 [14.1] years). Matched *BRCA1* and *BRCA2* SH carriers were less likely to present with higher disease severity than *BRCA1/BRCA2* DH carriers (*BRCA1*: OR 0.396 [0.180–0.87]; *BRCA2*: OR 0.224 [0.100–0.50]).
Conclusions: Multiple heterozygosity in HBOC core genes is more frequent than previously assumed. Our data indicate that gene-gene constellations can reshape tumour phenotype and clinical severity rather than following single-gene expectations. Integrating multiple pVs into risk assessment and counselling may enhance personalised surveillance and risk-reducing strategies for DH individuals and their families in the era of widespread multigene testing.

1. Introduction

Women with pathogenic or likely pathogenic variants (pVs) in breast cancer (BC) and ovarian cancer (OC) susceptibility genes face markedly elevated lifetime cancer risks [1–3]. Dependent on the gene involved and the carrier's sex, hereditary BC and/or OC (HBOC) may also increase risks of other cancers such as prostate and pancreatic cancer [4]. Disease penetrance and age-specific BC and OC incidences vary among genes [3], leading to their classification as highly penetrant genes (*BRCA1, BRCA2, PALB2, TP53*), moderately-to-highly penetrant genes (*CDH1, PTEN, STK11*), and moderately penetrant genes (*ATM, BARD1, BRIP1, CHEK2, RAD51C, RAD51D*) [3,5]. While genetic testing typically identifies no pV or a single pV, some individuals are found to harbour pVs in multiple genes associated with tumour predisposition syndromes (TPS), a phenomenon termed multiple heterozygosity or multi-locus inherited neoplasia allele syndrome [6].

Data on multiple heterozygous (MH) carriers are limited. Case reports, published in 1997, presented *BRCA1/BRCA2* double heterozygous (DH) individuals carrying Ashkenazi founder variants [7,8], followed by a series of reports worldwide [6,9–20]. A meta-analysis of 34 female *BRCA1/BRCA2* DH carriers of both Ashkenazi and non-Ashkenazi ancestry found no evidence of a more severe disease phenotype in DH carriers regarding age of BC/OC onset, cumulative risk for BC/OC, or probability of multiple primary cancers compared to single heterozygous (SH) carriers [11]. Similarly, an Israeli study of 15 female and seven male DH carriers within a cohort of 1191 *BRCA1* and/or *BRCA2* pV carriers showed no significant BC or OC rate differences between DH and SH carriers [12]. To address disease severity, Heidemann *et al.* developed a severity score based on the number of HBOC-associated cancers [13]. In a cohort of 8162 BC/OC at-risk families, the authors identified eight female DH carriers and pooled their data with published data, revealing a doubling of the mean severity score for DH carriers compared to their SH family members [13]. In contrast to *BRCA1* and *BRCA2*, data on DH carriers with pVs in at least one moderately penetrant gene (combined with another pV in a moderately or highly penetrant gene) remain scarce [15,18,21,22].

To address the limited understanding of multiple heterozygosity in HBOC, we took advantage of the German Consortium for HBOC (GC-HBOC) registry, one of the largest registries worldwide, documenting genotype-phenotype data of over 67,000 at-risk families [23]. As an interdisciplinary network comprising departments situated at 23 university hospitals in Germany and affiliated partners, GC-HBOC offers individuals genetic testing for pVs in a HBOC gene panel. To describe the prevalence and phenotype of MH carriers, we screened for MH carriers and compared the phenotype of DH individuals with SH carriers to characterize their clinical and genetic spectrum.

2. Methods

2.1. Cohort

Individuals were identified from the GC-HBOC registry, having consented to research participation according to ethically approved protocols at the respective centres. We included carriers of germline pVs in two or more of the following genes: HBOC core genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53*), genes previously implicated in HBOC but with disputed or potentially restricted, variant or population-specific pathogenicity (*FANCM, NBN*) [24], Lynch syndrome genes (*MLH1, MSH2, MSH6, PMS2*), or genes linked to other autosomal-dominant TPS. As control, we extracted SH carriers from the GC-HBOC registry. Unless specified otherwise, SH controls were tested for at least the 13 HBOC core genes (SH cohort). Further details are provided in the Supplementary Methods.

2.2. Disease severity

Female index DH and SH carriers were matched using 'MatchIT' v.4.5.5 [25] for genes involved, and age at last follow-up or first risk-reducing surgery (bilateral mastectomy, bilateral salpingo-oophorectomy), whichever occurred first. 'Matching without replacement' was selected. Index cases were defined as cancer patients tested by mutational search. Disease severity was assessed adapting a published scoring approach [13]. One point was counted for each HBOC-associated cancer (BC, contralateral BC, OC, pancreatic cancer), with the sum representing the severity score (range 1–4). For genes significantly associated with BC only, scoring was restricted to BC and contralateral BC (range 1–2).

2.3. Statistical analyses

Analyses were performed using *R* (https://www.r-project.org). Fisher exact tests and Kruskal-Wallis tests with Dunn post-hoc tests were used. For matched cohorts, we utilized Cumulative Link Mixed Model (CLMM) using 'ordinal' v.2023.12–4.1, with affected gene(s) as predictor and matched set as random effect. Post-hoc analysis used

'emmeans' v.1.10.6. Binary data were analysed using conditional logistic regression (CLR) with 'survival' v3.8.3. Statistics and assumptions were assessed using base R functions, 'ordinal', and 'survival' functions. Statistical significance was set at $p < 0.05$, with Benjamini-Hochberg (BH) correction for multiple testing [26].

3. Results

3.1. Prevalence of multiple heterozygosity

In the BRCA2006 registry with 26,983 carriers of HBOC pVs, 409 individuals from 346 families harboured pVs in two or more TPS genes (Table 1, Supplementary Figure 1). This MH cohort was predominately female (93%), with 73 different gene combinations identified. On average, 1.1 cancer diagnoses were documented per patient. A total of 400 individuals were DH (98%), with pVs in exactly two TPS-associated genes (Fig. 1). This cohort is termed DH cohort.

The SH all cohort included 26,574 individuals (92% women) from 16,440 families (Table 1), all identified with a single pV. Individuals of the SH all cohort had 0.9 cancers per person on average. Age at last follow-up and mean age at BC/OC diagnosis were similar between the SH all and MH cohorts (Table 1). The limited number of pancreatic and prostate cancers documented in the registry prevented comparing the age at diagnosis between cohorts.

Given the variation in TPS genes tested over time and with testing type (predictive cascade testing often restricted to the index person's pV (s)), we analysed individuals tested for at least the 13 HBOC core genes. In this subcohort, 3309 individuals had a single pV in one of the HBOC core genes (SH cohort), while 98 individuals carried pVs in two or more HBOC core genes, accounting for 2.9%. To address the potential impact of age-related somatic pVs in genes like *TP53* (*e.g.* clonal hematopoiesis of indeterminate potential), we examined *TP53* pV carriers further. No MH carrier tested for at least the 13 HBOC genes carried a *TP53* pV. Considering only SH cohort individuals with a cascade-test-confirmed *TP53* pV, 3263 SH individuals were identified. Excluding *TP53* carriers without confirmation by cascade testing, 2.9% of individuals carried pVs in two or more HBOC core genes.

3.2. Cancer burden

BC was the most common cancer in the DH cohort, diagnosed in 272 female and 6 male carriers (Fig. 1). Subsequent BC occurred in 69 female carriers (including two with three BCs) and one male carrier. Of these, 52 BCs (51 women, one man) were in the contralateral breast, and 20 BCs (all women) were in the same breast or of unknown side. To address uncertainty about recurrence *vs.* independent primary cancers, subsequent BCs in the same breast or of unknown side were excluded from the analyses. Sixty-eight DH carriers developed OC. Pancreatic cancer ($n = 2$) and prostate cancer ($n = 3$) were rarely reported. Therapeutic or risk-reducing mastectomy and risk-reducing bilateral salpingo-oophorectomy were documented in a minority of individuals (Fig. 1). Due to underrepresentation of male carriers and sex differences in cancer prevalence, subsequent analyses were restricted to female DH carriers.

3.3. BC phenotypes

In both the SH and DH cohorts, the hormone receptor (HR) and HER2 status of BC were assessed to determine if a second pV influences the BC phenotype (Fig. 2, Supplementary Tables 1,2). In the SH cohort, triple-negative BC (TNBC) was enriched in *BRCA1, BARD1, RAD51C*, and *RAD51D* SH carriers, while BC was predominantly HR-positive in *BRCA2, PALB2, TP53, ATM*, and *CHEK2* SH carriers, amongst others (Fig. 2A), which together defines a preferred BC phenotype dependent on the HBOC-gene involved. The DH cohort also showed preferential BC receptor phenotypes. For example, BCs were predominantly triple-

Table 1
Characteristics of the study cohorts.

	MH cohort (multiple heterozygotes)	SH all cohort (all controls)	SH cohort (HBOC core gene controls†)
Number of families*	346	16,440	3,248
n (patients)	409	26,574	3,309
(female/male/NA)	(380/29)	(24,401/2,169/4)	(3,247/60/2)
Single heterozygotes	NA	26,574 (24,401/2,169/4)	3,309 (3,247/60/2)
Double heterozygotes	400 (373/27)	NA	NA
Triple heterozygotes	9 (7/2)	NA	NA
Number of gene combinations and single genes affected	73	15	13
cancers per person [mean, (range)]	1.1 (0 – 4)	0.9 (0 – 6)	1.2 (0 – 5)
n (no cancer)	79 (19.3%)	9,259 (34.8%)	247 (7.5%)
n (1 cancer)	238 (58.2%)	12,766 (48.0%)	2,391 (72.3%)
n (2 cancers)	80 (19.6%)	3,767 (14.2%)	585 (17.7%)
n (3 cancers)	11 (2.7%)	672 (2.5%)	70 (2.1%)
n (4 cancers)	1 (0.2%)	89 (0.3%)	15 (0.5%)
n (5 cancers)	0 (0.0%)	16 (0.06%)	1 (0.03%)
n (6 cancers)	0 (0.0%)	5 (0.02%)	0 (0.0%)
Type of genetic testing			
mutational search	341 (83.4%)	16,341 (61.5%)	3,284 (99.2%)
predictive testing	68 (16.6%)	10,209 (38.4%)	25 (0.8%)
NA		24 (0.09%)	
Mean age at last follow-up ± SD	50.0 ± 13.6	49.1 ± 13.7	51.0 ± 12.6
Thereof:	Double heterozygotes (DH cohort)	Single heterozygotes	Single heterozygotes
Age at diagnosis of first breast cancer (mean, range)			
total	43.9 (20.0 – 85.5)	44.1 (17.5 – 92.3)	45.4 (21.5 – 92.3)
female	43.7 (20.0 – 85.5)	43.8 (17.5 – 92.3)	45.1 (21.5 – 92.3)
male	55.6 (43.2 – 60.5)	61.8 (33.1 – 83.4)	63.1 (44.2 – 83.4)
Age at diagnosis of ovarian/Fallopian tube/primary peritoneal cancer (mean, range)			
female	55.2 (29.5 – 85.1)	54.5 (5.5 – 92.5)	56.9 (19.5 – 84.6)
Age at diagnosis of pancreatic cancer (mean, range)			
total	67.8 (67.0 – 68.5)	55.5 (25.7 – 76.5)	57.5 (51.4 – 64.5)
female	67.8 (67.0 – 68.5)	54.4 (25.7 – 76.5)	54.0 (51.4 – 56.6)
male	NA	58.1 (36.5 – 76.5)	64.5
Age at diagnosis of prostate cancer (mean, range)			
male	55.7 (50.5 – 60.9)	65.3 (38.5 – 85.1)	61.1 (59.5 – 63.3)

*Families with at least one member harbouring two or more pVs (multiple heterozygotes) and one pV in TPS genes (single heterozygotes), respectively. †The SH cohort encompasses individuals who have undergone testing for at least the 13 core genes associated with HBOC and have been confirmed to carry a single pV associated with HBOC. This cohort is a subset of individuals comprising the SH all cohort shown in the middle. SD, standard deviation.

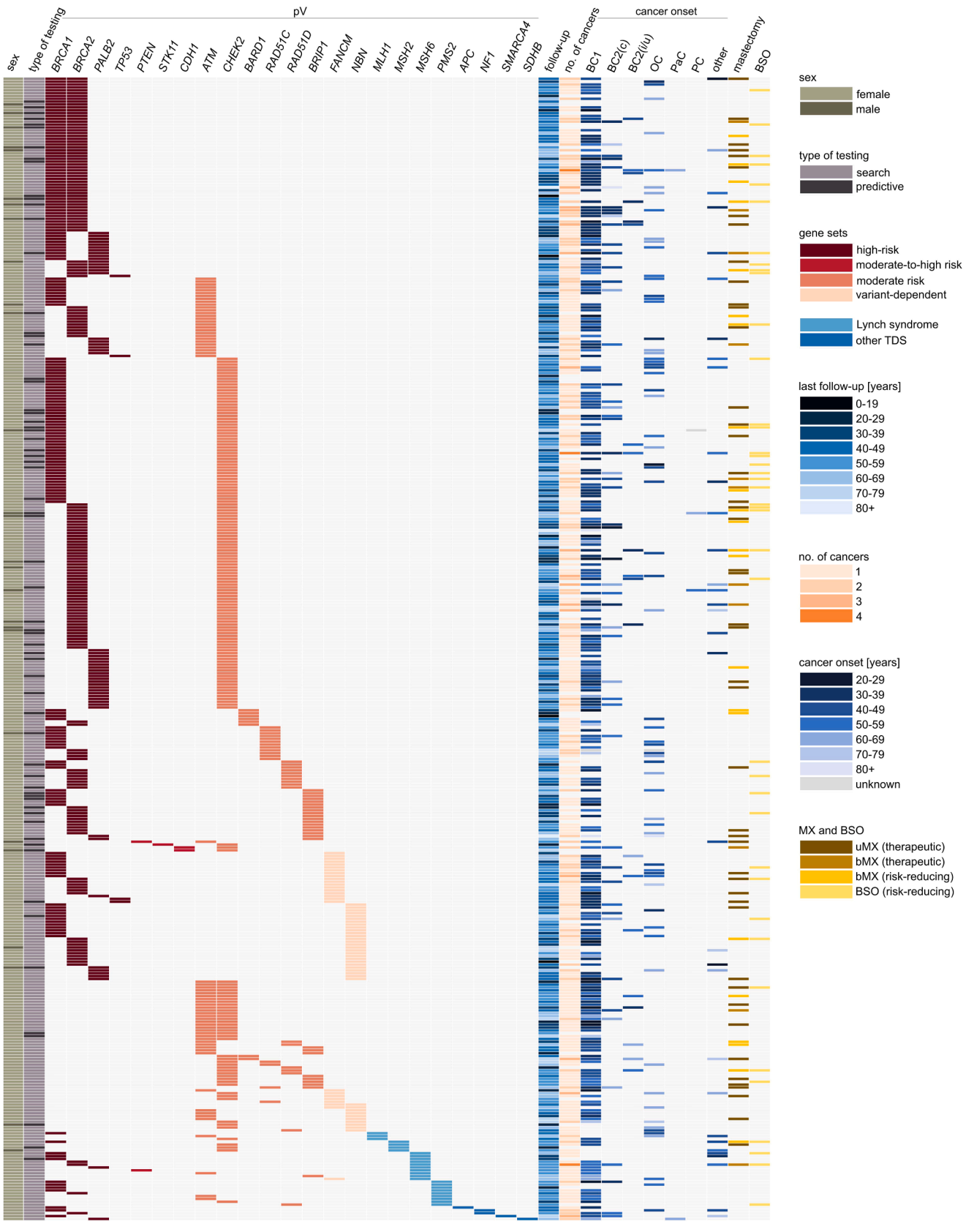
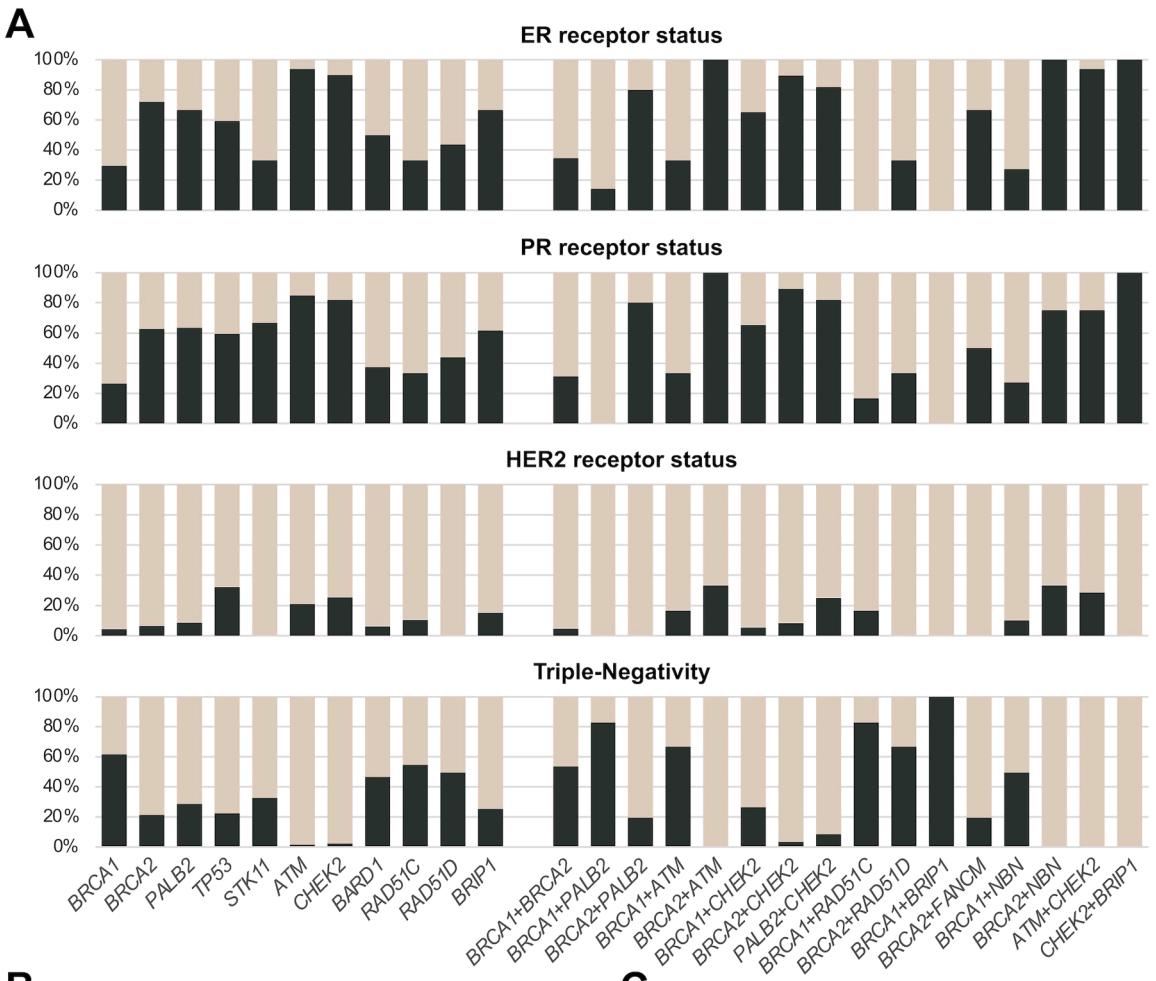


Fig. 1. Clinical and genetic characteristics of the DH cohort. Features are colour-encoded according to the legend shown on the right. pV, pathogenic variant; BC1, first breast cancer; BC2(c), second breast cancer, contralateral; BC2(i/u), second breast cancer in same breast or side unknown; OC, ovarian/Fallopian tube/peritoneal cancer; PaC, pancreatic cancer; PC, prostate cancer; uMX, unilateral mastectomy; bMX, bilateral mastectomy; BSO, bilateral salpingo-oophorectomy.



B

	HR	HER2
BRCA1+BRCA2 vs. BRCA1	$p = 1.000$	$p = 1.000$
BRCA1+BRCA2 vs. BRCA2	$\mathbf{p = 2.1 \times 10^{-3}}$	$p = 1.000$
BRCA1+PALB2 vs. BRCA1	$p = 0.957$	$p = 1.000$
BRCA1+PALB2 vs. PALB2	$\mathbf{p = 0.025}$	$p = 1.000$
BRCA1+ATM vs. BRCA1	$p = 1.000$	$p = 0.809$
BRCA1+ATM vs. ATM	$\mathbf{p = 2.1 \times 10^{-3}}$	$p = 1.000$
BRCA1+CHEK2 vs. BRCA1	$\mathbf{p = 8.8 \times 10^{-3}}$	$p = 1.000$
BRCA1+CHEK2 vs. CHEK2	$\mathbf{p = 0.018}$	$p = 0.521$
BRCA2+CHEK2 vs. BRCA2	$p = 0.368$	$p = 1.00$
BRCA2+CHEK2 vs. CHEK2	$p = 1.000$	$p = 0.521$

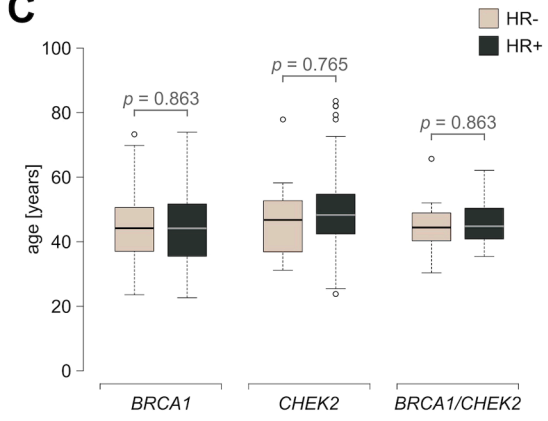


Fig. 2. HR and HER2 status of BC. (A) Fraction of BCs with a given phenotype in female carriers of HBOC-related pVs (dark columns, HR-positive and HER2-positive BC in the upper three plots and triple-negative BC in the lower plot, respectively). Single genes and gene combinations with $n < 3$ tumours were not considered in the analysis (Supplementary Table 1 for the number of BCs included in the analysis). For TNBC (bottom), triple-negative tumours were compared to non-TNBC tumours with at least one phenotype feature (ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2) being positive. **(B)** Comparison of the HR and HER2 status of BC using Fisher exact test of selected gene combinations (see Supplementary Table 2 for all combinations tested). Obtained *p*-values were adjusted for multiple testing using the Benjamini-Hochberg method. Significant *p*-values are in bold. **(C)** Age at diagnosis of HR-negative and HR-positive BC (legend) in female carriers of a single *BRCA1* pV, single *CHEK2* pV, and double *BRCA1/CHEK2* pVs. Individuals may present with multiple BC manifestations (number of cancers analysed are given in Supplementary Table 3). *P*-values were determined using the Kruskal-Wallis test, followed by Dunn post-hoc test and Benjamini-Hochberg correction.

negative in individuals carrying pVs in *BRCA1/BRCA2*, *BRCA1/PALB2*, *BRCA1/ATM*, *BRCA1/RAD51C* or *BRCA1/BRIP1* (Fig. 2A).

No significant differences in the HER2 status were observed between female SH and DH cohorts (BH-adjusted $p = 0.521$–1.000; Fig. 2B, Supplementary Table 2). A *BRCA1* pV combined with a pV in most HBOC-associated genes favoured a triple-negative BC phenotype due to a lower rate of HR-positivity, regardless of whether the pV in the second (non-*BRCA1*) HBOC-related gene is associated with HR-positivity or not, when isolated: BCs in *BRCA1/BRCA2* DH carriers showed a significantly lower rate of HR-positivity compared to *BRCA2* SH carriers (BH-

adjusted $p = 2.1 \times 10^{-3}$), as did *BRCA1/ATM* DH *vs. ATM* SH (BH-adjusted $p = 2.1 \times 10^{-3}$) and *BRCA1/PALB2* DH *vs. PALB2* SH carriers (BH-adjusted $p = 0.025$).

In contrast, TNBC was less frequently observed in *BRCA1/CHEK2* DH carriers, with HR-positive cancers in about two thirds of BCs (Fig. 2A). HR-positivity was significantly higher in *BRCA1/CHEK2* DH carriers compared to *BRCA1* SH carriers (BH-adjusted $p = 8.8 \times 10^{-3}$; Fig. 2B), but did not fully align with that observed in *CHEK2* SH carriers (BH-adjusted $p = 0.018$).

Given that HR-positivity might relate to the age at BC diagnosis, we examined age differences between HR-negative and HR-positive BCs. This analysis included all BCs with available HR status, regardless of whether it was the first BC or a subsequent contralateral tumour. No statistically significant age differences were observed in any group. In *BRCA1* SH carriers, the median age (interquartile range [IQR]) at diagnosis for HR-negative and HR-positive BC was 44.2 [13.6] and 44.2 [16.2] years, respectively (BH-adjusted $p = 0.863$; Fig. 2C, Supplementary Table 3). For *CHEK2* SH carriers, these values were 46.8 [15.2] and 48.3 [12.4] years (BH-adjusted $p = 0.765$). Also, in *BRCA1/CHEK2* DH carriers, median ages were similar: 44.4 [8.6] and 44.8 [8.7] years for HR-negative and HR-positive BC, respectively (BH-adjusted $p = 0.863$).

Individuals with a *BRCA2* pV combined with another HBOC-associated pV showed low triple-negativity rates (except *BRCA1/BRCA2* and *BRCA2/RAD51D* DH carriers), aligning with BCs in *BRCA2* SH carriers (Fig. 2A,B). These tumours were predominantly HR-positive and HER2-negative. *ATM/CHEK2* DH carriers developed no TNBC, consistent with *ATM* or *CHEK2* SH carriers (Fig. 2A).

3.4. Age of cancer onset

BC onset ranged from ~20 to > 90 years, with a median age at first BC diagnosis in the 40's for most genes in the SH cohort (Fig. 3A). In the SH cohort, *TP53* carriers had a median age of first BC diagnosis at 39.6 years [13.1], followed by *BRCA1* SH carriers (41.3 [14.1]; Supplementary Table 4). In the DH cohort, *BRCA1/PALB2* and *BRCA1/BRCA2* DH carriers developed first BC at median ages of 33.5 [15.8] and 36.9 years [10.1], followed by *BRCA1/BRIP1* (37.6 [9.2]), and *BRCA2/ATM* (40.1 [12.1]) DH carriers, amongst others.

Differences in age of first BC between SH and DH carriers did not reach statistical significance (BH-adjusted $p > 0.05$), except for *BRCA1/BRCA2* DH *vs. BRCA2* SH carriers (BH-adjusted $p = 0.002$). However, the comparison between *BRCA1/BRCA2* DH and *BRCA1* SH carriers (where *BRCA1* is the more penetrant gene) was not significant (BH-adjusted $p = 0.199$).

For certain gene combinations, particularly those involving pVs in two moderately penetrant genes, our data suggested a trend toward a reduced age of onset. For instance, *ATM/CHEK2* DH carriers had a median BC onset age of 41.5 years [14.0], compared to *ATM* SH (46.1 [13.2]) or *CHEK2* SH (47.6 [14.9]; Supplementary Table 4) carriers. The age range of BC onset in *ATM/CHEK2* DH individuals falls within the range observed in *BRCA1* SH carriers (41.3 [14.1]; Fig. 3A).

Of 771 patients with OC in SH and DH cohorts, median age at OC diagnosis was 56.5 years in SH and 55.8 years in DH carriers. In SH carriers, median OC diagnosis age was 53.7 [11.6] for *BRCA1*, 59.5 [12.4] for *BRCA2*, and 56.5 [20.5] years for *PALB2* (Fig. 3B, Supplementary Table 4). DH carriers with pVs in *BRCA1* plus another HBOC core gene had median OC diagnosis ages of 50.0 [12.9] for *BRCA1/CHEK2*, 54.0 [7.6] for *BRCA1/ATM*, and 58.2 [13.0] years for *BRCA1/BRCA2*. Differences in age at OC diagnosis between SH and DH

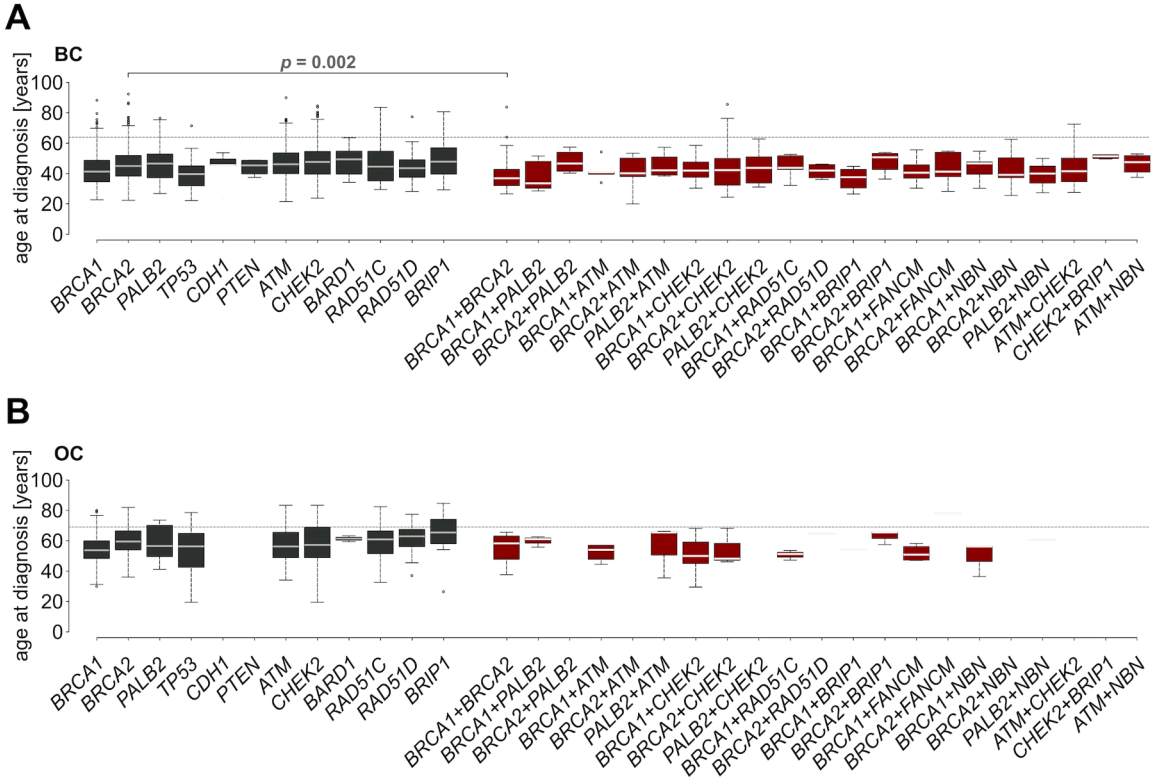


Fig. 3. Age at diagnosis of BC and OC in carriers of pVs in HBOC-associated genes. Age distribution of female patients with **(A)** first BC diagnosis ($n \geq 3$), and **(B)** OC diagnosis, if appropriate. The median age of women diagnosed with BC (65 years) and OC (68 years) in Germany is indicated by a dashed line in (A) and (B), respectively. Statistical significance of HBOC core gene combinations was assessed using Kruskal-Wallis test, followed by Dunn post-hoc test and Benjamini-Hochberg adjustment, if appropriate. Numbers of BCs and OCs included in the analysis together with the median age at cancer diagnosis and IQR are listed in Supplementary Table 4.

carriers did not reach statistical significance (BH-adjusted $p > 0.05$).

3.5. Severity score

Finally, we investigated disease severity using a scoring system based on HBOC-associated cancers (BC, contralateral BC, OC, and pancreatic cancer, Fig. 4A,B). Female DH and SH cancer patients tested by mutational search were aged-matched to account for differences in follow-up and risk-reducing surgery. CLMM analysis ($p = 4.65 \times 10^{-4}$, likelihood ratio test) indicated carriers of single *BRCA1* or *BRCA2* pVs had lower odds of being in higher severity scores compared to *BRCA1/BRCA2* DH carriers (*BRCA1*: OR 0.396, 95%CI 0.180–0.87, $p = 0.021$; *BRCA2*: OR 0.224, 95%CI 0.100–0.50, $p = 2.82 \times 10^{-4}$; Supplementary Table 5). Post-hoc tests confirmed significant differences (*BRCA1*: $p = 0.021$; *BRCA2*: $p = 8 \times 10^{-4}$, BH-adjusted). CLR with binarized severity scores (1 *vs.* >1) support these results (*BRCA1*: OR 0.44, 95%CI 0.200–0.97, $p = 0.042$; *BRCA2*: OR 0.253, 95%CI 0.113–0.57, $p = 8.66 \times 10^{-4}$).

For gene combinations with restricted associated cancer spectra (*ATM*, *CHEK2*; Fig. 4B), severity scores were adjusted based on overlapping cancer spectra, focusing on BC alone (Fig. 4C). CLR showed significance for *BRCA1/CHEK2* DH vs. *CHEK2* SH carriers (OR 0.309, 95%CI 0.127–0.75, $p = 9.69 \times 10^{-3}$, Supplementary Table 5), but not for *BRCA1/CHEK2* DH *vs.* *BRCA1* SH carriers ($p = 0.145$). Other combinations were not significant.

4. Discussion

Genetic testing for pVs linked to TPS can inform clinical decisions regarding therapy, risk-reducing surgeries, and surveillance for index patients and at-risk relatives [1,27]. This study characterizes cancers in DH individuals diagnosed with HBOC and presents results from a national MH cohort, including gene combinations rarely reported before. Collectively, our data suggest that the phenotype can, dependent on the gene combination, be modified by the presence of a second pV.

Our findings support that a given pV influences HR and HER2 status in BC. This is well established for SH carriers; *e.g.* *BRCA1* SH carriers preferentially develop TNBC [28,29], particularly if diagnosed before age 50 [30], while *BRCA2* SH carriers typically present with HR-positive BC [3]. In individuals with a *BRCA1* pV and a pV in second HBOC-associated gene, BC predominantly appears as triple-negative in the majority of gene combinations studied, even when the second pV favours HR-positive BC in SH carriers. Thus, we conclude that *BRCA1* pVs dominate the HR phenotype in most HBOC-associated pV combinations. However, *BRCA1/CHEK2* DH carriers showed increased HR-positivity compared to *BRCA1* SH individuals. In SH carriers, *CHEK2* pVs is enriched in HR-positive BC [31], which aligns with our control cohort. Previous data on *BRCA1/CHEK2* DH carriers is limited [15,18,21,32]; of eleven reported cases with available BC phenotype, nine were HR-positive [15,18,32]. Notably, our study found no significant differences in the age at which *BRCA1/CHEK2* DH carriers were diagnosed

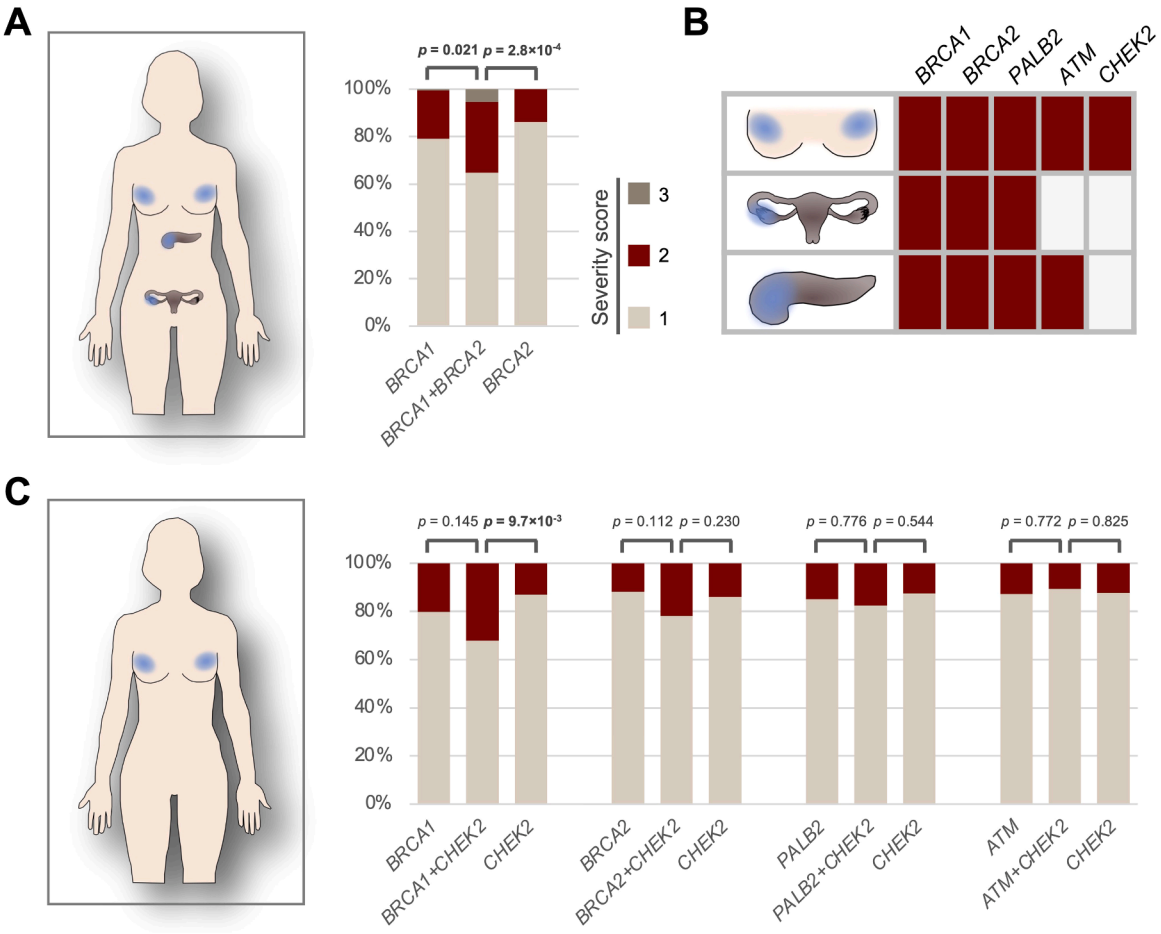


Fig. 4. Severity score for female carriers of pVs in HBOC-associated genes. (A) Stacked bar plot showing the severity score of female *BRCA1* SH, *BRCA2* SH and *BRCA1/BRCA2* DH carriers with four relevant cancer types (BC, contralateral BC, OC, pancreatic cancer; diagram on the left). The score is color-coded as indicated in the legend. **(B)** Cancer predisposition profile indicated by red boxes for selected genes associated with HBOC. **(C)** For gene combinations with a restricted cancer predisposition profile, the severity score was adjusted to include only overlapping cancers, specifically breast cancers. *P*-values are derived from a cumulative link mixed model for ordinal data (A) and conditional logistic regression for binary data (C), both considering the matched cohort structure in the respective models (Supplementary Table 5).

with HR-positive *vs.* HR-negative BC, suggesting age at diagnosis is not the main factor contributing to the increased frequency of HR-positivity. Our results thus support a phenotypic interaction between the *BRCA1/CHEK2* pVs, suggesting that this interplay may enhance the dominance of the *CHEK2* HR phenotype. A recent study analysing genomes of cancers in patients with biallelic germline *CHEK2* pVs found that these tumours lack homologous recombination deficiency (HRD) markers typically associated with *BRCA1/2*-deficient cancers [22]. Thus, further research is needed to determine the HRD phenotype of *BRCA1/CHEK2* DH BCs.

Current HBOC guidelines do not specify whether recommendations for female SH carriers extend to DH carriers. Our data indicate that age at BC diagnosis in female DH carriers often mirrors that of carriers with a single pV in the more penetrant gene. This observation suggests that for most gene combinations, intensified surveillance could begin at the age recommended for carriers of a single pV in the more penetrant gene. However, carriers of specific gene combinations like *ATM/CHEK2* showed a median BC onset age similar to carriers of a single *BRCA1* pV, in line with recent data [32]. In Germany, intensified breast surveillance typically starts at 25 years for female *BRCA1* SH carriers and 30 years for female *ATM* and *CHEK2* SH carriers. Our data suggest that earlier surveillance for individuals with specific gene combinations may be beneficial. However, our analysis did not reach statistical significance, likely due to limited case numbers and investigation in larger cohorts is necessary to validate these findings and optimize personalized surveillance for DH individuals. The higher severity score could be considered in clinical recommendations regarding risk-reducing surgery for female *BRCA1/BRCA2* DH carriers.

For male DH carriers, the limited number of documented cases makes it challenging to draw conclusions about their phenotype. Our preliminary analysis suggests earlier cancer onset. Larger prospective studies are required to address clinical recommendations for DH carriers comprehensively.

Epidemiologically, pV carrier rates vary by population, and there is evidence that the phenotype of DH carriers might be altered by the ethnic background. In the Ashkenazi population [33], ~3% carry *BRCA1* or *BRCA2* pVs, mostly linked to three founder variants [34–36]. Among female Ashkenazi cancer patients, ~10% of BC and ~30% of OC cases are associated with *BRCA1* or *BRCA2* pVs [33]. The high prevalence of SH carriers in the Ashkenazi population is expected to result in an increased frequency of DH carriers within this group.

Earlier reports on Ashkenazi families did not support a more severe phenotype in DH carriers *vs.* SH carriers [11,12]. Asian DH individuals showed similar BC phenotypes and onset ages to European cohorts (26–55 years). Previous studies across various populations of DH individuals reported first BC between 23–70 years and OC between 36–66 years [11,13,14,37]. Here, our study shows DH carriers in the German population can exhibit more severe disease phenotypes than SH carriers. Population-specific differences may be influenced by genetic modifiers like single nucleotide polymorphisms [38], and non-genetic factors such as environment and lifestyle including reproduction, diet, and body mass index. Some of these factors may serve as targets for prevention [39].

Moreover, our data indicate multiple heterozygosity is more prevalent than previously thought, affecting ~3% of carriers with HBOC-associated pVs. This is a lower bound estimate, considering only the 13 core HBOC genes.

In conclusion, our findings support disease spectrum modification in DH individuals with HBOC across certain gene combinations. Future molecular tumour analyses may reveal mechanisms driving phenotype modification [40]. As clinical management recommendations depend on pV detection [41–43], careful pedigree analysis for TPS in the other family lineage is essential, particularly for predictive cascade testing. Cascade testing for pV(s) identified in the index patient may leave additional pVs undetected, potentially missing opportunities for adapting clinical management. Recent data from panel sequencing in cascade testing suggest that unforeseen findings influencing clinical management can be detected in approximately 2% of tested relatives, including those in whom the familial pV was excluded [44]. The findings reported herein mark a step towards developing clinical evidence-based guidelines tailored to the growing group of MH carriers. Identification of additional MH individuals and families, along their structured follow-up, will inform future recommendations and personalized approaches in genetic testing and clinical care.

Data sharing

Data of this study on germline variants and classification are made available through submission to the ClinVar database. As some data could potentially lead to patient identification, individual data of patients cannot be made available. Aggregated data are available from the corresponding author upon reasonable requests including submission of a brief research proposal. Necessary data sharing agreements will need to be completed.

CRediT authorship contribution statement

Jan Hauke: Resources, Writing – review & editing. **Tanja Fehm:** Resources, Writing – review & editing. **Cornelia Meisel:** Resources, Writing – review & editing. **Marion Kiechle:** Resources, Writing – review & editing. **Bernhard H.F. Weber:** Resources, Writing – review & editing. **Andrea Gehrig:** Resources, Writing – review & editing. **Christine Solbach:** Resources, Writing – review & editing. **Tim Ripperger:** Resources, Writing – review & editing. **Bjoern Sander:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **Dorothee Speiser:** Resources, Writing – review & editing. **Barbara Wappenschmidt:** Resources, Writing – review & editing. **Christoph Engel:** Resources, Validation, Writing – review & editing. **Bahriye Aktas:** Resources, Writing – review & editing. **Susanne Ledig:** Resources, Writing – review & editing. **Alexander E. Volk:** Resources, Writing – review & editing. **Nicola Dikow:** Resources, Writing – review & editing. **Christopher Schroeder:** Resources, Writing – review & editing. **Norbert Arnold:** Resources, Writing – review & editing. **Tobias Blaum:** Resources, Writing – review & editing. **Nadia Harbeck:** Resources, Writing – review & editing. **Matthias Rath:** Resources, Writing – review & editing. **Elena Leinert:** Resources, Writing – review & editing. **Pauline Wimberger:** Resources, Writing – review & editing. **Rita Schmutzler:** Funding acquisition, Resources, Writing – review & editing. **Britta Blümcke:** Resources, Writing – review & editing. **Kerstin Rhiem:** Resources, Writing – review & editing. **Julia Ritter:** Resources, Writing – review & editing. **Bernd Auber:** Resources, Writing – review & editing. **Nina Ditsch:** Resources, Writing – review & editing. **Eric Hahnen:** Data curation, Investigation, Resources, Writing – review & editing. **Susanne Morlot:** Conceptualization, Resources, Writing – review & editing. **Monika Maringa:** Resources, Writing – review & editing. **Anna Hester:** Resources, Writing – review & editing. **Anke Waha:** Resources, Writing – review & editing. **Jörg Schröder:** Resources, Writing – review & editing. **Natalie Herold:** Data curation, Investigation, Resources, Writing – original draft, Writing – review & editing. **Monika M. Golas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jäger Bernadette A. S.:** Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Bahriye Aktas – Honoraria and/or Consulting or Advisory Role: Amgen, AstraZeneca, Daiichi-Sankyo, Eisai, Gilead, GSK, Lilly, Medtronic, MSD, Novartis, Onkowissen, Pfizer, Roche, Seagen, Stemline,

Tesaro.

Tobias Blaum – Conference stipend: Lilly Pharma.

Nina Ditsch – Advisory Boards: Gilead, Lilly, MSD, Novartis, Pfizer, Roche, Exact Sciences, Merit-Medical, Pierre-Fabre; Lectures and speakers bureaus: AstraZeneca, Fa. Aurikamed, COCS GmbH, Daiichi-Sankyo, ESMO International, Novartis, Roche, Lilly, Pfizer, Gilead, Novartis, Onkowissen, Jörg Eickeler Kongress, if-Kongress, Fa. Titanium Textiles, RG Kongress, Onkowissen, ClinSol GmbH, Kelcon GmbH, Dt. Apotheker Verlag, Fa. Menarini-Stemline, FA. KUKM Kongress, Fa. BMMC Kongress, Fa. Gapinsaat, VHS Augsburg, Fa. Medi-Seminar GmbH, RRC-Kongress, I-Med. Institute Berlin; manuscript: pfm Medical ag; trial funding: Gilead, BZKF, pfm Medical ag; co-author: Lilly (MonarchE-study, Senology 2026, DKK 2026); Astrazeneca (Olympia-N study; SABCS 2025).

Tanja Fehm – Honoraria: Medconcept, Onkowissen, FOMF, Aurikamed; Travel Grants: Roche, Daichii Sankyo, Pfizer, Novartis.

Monika Golas – Conference support: German Society for Senology, honoraria from Dt. Ärzteblatt.

Eric Hahnen – Co-inventor of a patent "Method for assessing homologous recombination deficiency in ovarian cancer cells" (EP4141127B1, WO/2023/031121) issued outside the submitted work.

Nadia Harbeck – Honoraria and/or Consulting or Advisory Role: AstraZeneca, Daiichi-Sankyo, Exact Sciences, Gilead, Lilly, MEDSCAPE, Menarini-Stemline, MSD, Novartis, Pierre-Fabre, Pfizer, Roche, Springer, Viatris, Zuelligpharma. Other: Co-Director WSG (Women's Cancer Study Group).

Jan Hauke – Honoraria/Lecture fee: AstraZeneca.

Natalie Herold – Conference support: German Cancer Congress.

Anna Hester – Honoraria and/or Consulting or Advisory Role or Conference participation: Roche, Pfizer, Gilead, Sandoz, Ismar Healthcare NV-SA. Currently employed at Boehringer Ingelheim.

Bernadette A. S. Jäger – Honoraria and/or Consulting or Advisory Role: AstraZeneca, OnkoZert.

Marion Kiechle – Renumeration: Springer Press, Biermann Press, Celgene, Astra Zeneca, Myriad Genetics, TEVA, Eli Lilly, GSK, Seagen, AllergoSan, NutriImmun, FOMF, Jenapharm, Roche, BESINS, Bayer AG, Novartis, Pierre Fabre, Abbvie. Consultant/advisory role: Myriad Genetics, Bavarian KVB, Roche, DKMS Life, BLÄK, TEVA, Exeltis, BESINS, Bayer AG, Eurobio-scientific, Magna Med Group. Equity owner: AIM GmbH, In-Manas GmbH, Therawis Diagnostic GmbH. Funding: Sphingotec, Deutsche Krebshilfe, DFG, Senator Roesner Foundation, Dr. Pommer-Jung Foundation, Waltraut Bergmann Foundation, Bavarian State Ministry of Economy, BMBF, Innovation Fond GBA, Bavarian State Ministry of Health and Prevention.

Susanne Morlot – Honoraria: AstraZeneca.

Matthias Rath – Honoraria/Lecture fee: AstraZeneca.

Kerstin Rhiem - Honoraria by Alexion, Pfizer, AstraZeneca; Advisory Role: Jenapharm, AstraZeneca.

Rita Schmutzler – Honoraria by MSD, AstraZeneca/Merck. Co-inventor of a patent "Method for assessing homologous recombination deficiency in ovarian cancer cells" (EP4141127B1, WO/2023/031121) issued outside the submitted work.

Christopher Schroeder – Institutional grants: Novartis, Illumina; grants: BMS Stiftung Immunonkologie.

Christine Solbach – Lecture/speaker engagement fees: Dialog Service GmbH, Jörg Eickeler, MedConcept, Medela, Samsung, Novartis, Roche, Pfizer, LÄKH.

Pauline Wimberger – Research funding for institution from Amgen, abbvie, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma, Clovis, Lilly, honoraria from Amgen, abbvie, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma, Clovis, TEVA, Eisai, Lilly, Gilead, Daichii Sankyo. PW participates at advisory boards from Amgen, abbvie, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma, Clovis, TEVA, Eisai, Lilly, Gilead and Daichii Sankyo.

All remaining authors have declared no conflicts of interest.

Acknowledgement

The GC-HBOC was supported by the German Cancer Aid (grant no 110837 and grant no 70114178, coordinator: Rita K. Schmutzler, Cologne) and the Federal Ministry of Education and Research (BMBF), Germany (grant no 01GY1901). The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript; nor in the decision to submit the manuscript for publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2026.116874.

References

[1] Kast K, Rhiem K, Wappenschmidt B, Hahnen E, Hauke J, et al. Prevalence of BRCA1/2 germline mutations in 21401 families with breast and ovarian cancer. J Med Genet 2016;53:465–71. https://doi.org/10.1136/jmedgenet-2015-103672.

[2] Harter P, Hauke J, Heitz F, Reuss A, Kommoss S, et al. Prevalence of deleterious germline variants in risk genes including BRCA1/2 in consecutive ovarian cancer patients (AGO-TR-1). PLOS ONE 2017;12:e0186043. https://doi.org/10.1371/journal.pone.0186043.

[3] Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, et al. Breast cancer risk genes - association analysis in more than 113,000 women. N Engl J Med 2021; 384:428–39. https://doi.org/10.1056/NEJMoa1913948.

[4] Silvestri V, Leslie G, Barnes DR, Agnarsson BA, Aittomäki K, et al. Characterization of the Cancer Spectrum in Men With Germline BRCA1 and BRCA2 Pathogenic Variants: Results From the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). JAMA Oncol 2020;6:1218–30. https://doi.org/10.1001/jamaoncol.2020.2134.

[5] Rhiem K, Auber B, Briest S, Dikow N, Ditsch N, et al. Consensus Recommendations of the German Consortium for Hereditary Breast and Ovarian Cancer. Breast Care (Basel) 2022;17:199–207. https://doi.org/10.1159/000516376.

[6] McGuigan A, Whitworth J, Andreou A, Hearn T, Tischkowitz M, et al. Multilocus Inherited Neoplasia Allele Syndrome (MINAS): an update. Eur J Hum Genet 2022; 30:265–70. https://doi.org/10.1038/s41431-021-01013-6.

[7] Ramus SJ, Friedman LS, Gayther SA, Ponder BA, Bobrow L, et al. A breast/ovarian cancer patient with germline mutations in both BRCA1 and BRCA2. Nat Genet 1997;15:14–5. https://doi.org/10.1038/ng0197-14.

[8] Gershoni-Baruch R, Dagan E, Kepten I, Freid G. Co-segregation of BRCA1 185delAG mutation and BRCA2 6174delT in one single family. Eur J Cancer 1997;33:2283–4. https://doi.org/10.1016/s0959-8049(97)00223-2.

[9] Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol 2002;20:1480–90. https://doi.org/10.1200/jco.2002.20.6.1480.

[10] Caldes T, de la Hoya M, Tosar A, Sulleiro S, Godino J, et al. A breast cancer family from Spain with germline mutations in both the BRCA1 and BRCA2 genes. J Med Genet 2002;39:e44. https://doi.org/10.1136/jmg.39.8.e44.

[11] Leegte B, van der Hout AH, Deffenbaugh AM, Bakker MK, Mulder IM, et al. Phenotypic expression of double heterozygosity for BRCA1 and BRCA2 germline mutations. J Med Genet 2005;42:e20. https://doi.org/10.1136/jmg.2004.027243.

[12] Lavie O, Narod S, Lejbkowicz F, Dishon S, Goldberg Y, et al. Double heterozygosity in the BRCA1 and BRCA2 genes in the Jewish population. Ann Oncol 2011;22: 964–6. https://doi.org/10.1093/annonc/mdq460.

[13] Heidemann S, Fischer C, Engel C, Fischer B, Harder L, et al. Double heterozygosity for mutations in BRCA1 and BRCA2 in German breast cancer patients: implications on test strategies and clinical management. Breast Cancer Res Treat 2012;134: 1229–39. https://doi.org/10.1007/s10549-012-2050-4.

[14] Rebbeck TR, Friebel TM, Mitra N, Wan F, Chen S, et al. Inheritance of deleterious mutations at both BRCA1 and BRCA2 in an international sample of 32,295 women. Breast Cancer Res 2016;18:112. https://doi.org/10.1186/s13058-016-0768-3.

[15] Sukumar J, Kassem M, Agnese D, Pilarski R, Ramaswamy B, et al. Concurrent germline BRCA1, BRCA2, and CHEK2 pathogenic variants in hereditary breast cancer: a case series. Breast Cancer Res Treat 2021;186:569–75. https://doi.org/10.1007/s10549-021-06095-w.

[16] Le Page C, Rahimi K, Rodrigues M, Heinzelmann-Schwarz V, Recio N, et al. Clinicopathological features of women with epithelial ovarian cancer and double heterozygosity for BRCA1 and BRCA2: A systematic review and case report analysis. Gynecol Oncol 2020;156:377–86. https://doi.org/10.1016/j.ygyno.2019.11.019.

[17] Sepahi I, Faust U, Sturm M, Bosse K, Kehrer M, et al. Investigating the effects of additional truncating variants in DNA-repair genes on breast cancer risk in BRCA1-positive women. BMC Cancer 2019;19:787. https://doi.org/10.1186/s12885-019-5946-0.

[18] Sokolenko AP, Bogdanova N, Kluzniak W, Preobrazhenskaya EV, Kuligina ES, et al. Double heterozygotes among breast cancer patients analyzed for BRCA1, CHEK2, ATM, NBN/NBS1, and BLM germ-line mutations. Breast Cancer Res Treat 2014; 145:553–62. https://doi.org/10.1007/s10549-014-2971-1.

[19] Laish I, Friedman E, Levi-Reznick G, Kedar I, Katz L, et al. Double heterozygotes of BRCA1/BRCA2 and mismatch repair gene pathogenic variants: case series and clinical implications. Breast Cancer Res Treat 2021;188:685–94. https://doi.org/10.1007/s10549-021-06258-9.
[20] Noh JM, Choi DH, Nam SJ, Lee JE, Kim JW, et al. Characteristics of double heterozygosity for BRCA1 and BRCA2 germline mutations in Korean breast cancer patients. Breast Cancer Res Treat 2012;131:217–22. https://doi.org/10.1007/s10549-011-1718-5.
[21] Turnbull C, Seal S, Renwick A, Warren-Perry M, Hughes D, et al. Gene-gene interactions in breast cancer susceptibility. Hum Mol Genet 2012;21:958–62. https://doi.org/10.1093/hmg/ddr525.
[22] Hinic S, van der Post RS, Vreede L, Schuurs-Hoeijmakers J, Koene S, et al. The genomic landscape of breast and non-breast cancers from individuals with germline CHEK2 deficiency. JNCI Cancer Spectr 2024;8. https://doi.org/10.1093/jncics/pkae044.
[23] Schmutzler RK. Quality and Quantity: How to Organize a Countrywide Genetic Counseling and Testing. Breast Care (Basel) 2021;16:196–201. https://doi.org/10.1159/000515429.
[24] Figlioli G, Bogliolo M, Catucci I, Caleca L, Lasheras SV, et al. The FANCM:p. Arg658* truncating variant is associated with risk of triple-negative breast cancer. NPJ Breast Cancer 2019;5:38. https://doi.org/10.1038/s41523-019-0127-5.
[25] Ho D, Imai K, King G, Stuart EA. MatchIt: Nonparametric Preprocessing for Parametric Causal Inference. J Stat Softw 2011;42:1–28. https://doi.org/10.18637/jss.v042.i08.
[26] Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B (Methodol) 1995;57:289–300.
[27] Desmond A, Kurian AW, Gabree M, Mills MA, Anderson MJ, et al. Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment. JAMA Oncol 2015;1:943–51. https://doi.org/10.1001/jamaoncol.2015.2690.
[28] Engel C, Rhiem K, Hahnen E, Loibl S, Weber KE, et al. Prevalence of pathogenic BRCA1/2 germline mutations among 802 women with unilateral triple-negative breast cancer without family cancer history. BMC Cancer 2018;18:265. https://doi.org/10.1186/s12885-018-4029-y.
[29] Rhiem K, Zachariae S, Waha A, Grill S, Hester A, et al. Prevalence of Pathogenic Germline Variants in Women with Non-Familial Unilateral Triple-Negative Breast Cancer. Breast Care (Basel) 2023;18:106–12. https://doi.org/10.1159/000528972.
[30] Vaziri SA, Krumroy LM, Elson P, Budd GT, Darlington G, et al. Breast tumor immunophenotype of BRCA1-mutation carriers is influenced by age at diagnosis. Clin Cancer Res 2001;7:1937–45.
[31] Hauke J, Horvath J, Groß E, Gehrig A, Honisch E, et al. Gene panel testing of 5589 BRCA1/2-negative index patients with breast cancer in a routine diagnostic setting: results of the German Consortium for Hereditary Breast and Ovarian Cancer. Cancer Med 2018;7:1349–58. https://doi.org/10.1002/cam4.1376.
[32] Agaoglu NB, Bychkovsky BL, Horton C, Lo MT, Polfus L, et al. Cancer burden in individuals with single versus double pathogenic variants in cancer susceptibility genes. Genet Med Open 2024;2:101829. https://doi.org/10.1016/j.gimo.2024.101829.
[33] Rennert G, Bisland-Naggan S, Barnett-Griness O, Bar-Joseph N, Zhang S, et al. Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. N Engl J Med 2007;357:115–23. https://doi.org/10.1056/NEJMoa070608.
[34] Struewing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM, et al. The carrier frequency of the BRCA1 185delAG mutation is approximately 1 % in Ashkenazi Jewish individuals. Nat Genet 1995;11:198–200. https://doi.org/10.1038/ng1095-198.
[35] Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nat Genet 1996;14:185–7. https://doi.org/10.1038/ng1096-185.
[36] Oddoux C, Struewing JP, Clayton CM, Neuhausen S, Brody LC, et al. The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. Nat Genet 1996;14:188–90. https://doi.org/10.1038/ng1096-188.
[37] Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. Jama 2017;317:2402–16. https://doi.org/10.1001/jama.2017.7112.
[38] Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. Am J Hum Genet 2019;104:21–34. https://doi.org/10.1016/j.ajhg.2018.11.002.
[39] Kiechle M, Dukatz R, Yahiaoui-Doktor M, Berling A, Basrai M, et al. Feasibility of structured endurance training and Mediterranean diet in BRCA1 and BRCA2 mutation carriers - an interventional randomized controlled multicenter trial (LIBRE-1). BMC Cancer 2017;17:752. https://doi.org/10.1186/s12885-017-3732-4.
[40] Kuhlen M, Hofmann TG, Golas MM. Puzzling phenomenon: adult-onset cancer predisposition and pediatric cancer. Trends Cancer 2024;10:481–5. https://doi.org/10.1016/j.trecan.2024.02.011.
[41] Bick U, Engel C, Krug B, Heindel W, Fallenberg EM, et al. High-risk breast cancer surveillance with MRI: 10-year experience from the German consortium for hereditary breast and ovarian cancer. Breast Cancer Res Treat 2019;175:217–28. https://doi.org/10.1007/s10549-019-05152-9.
[42] Engel C, Fischer C, Zachariae S, Bucksch K, Rhiem K, et al. Breast cancer risk in BRCA1/2 mutation carriers and noncarriers under prospective intensified surveillance. Int J Cancer 2020;146:999–1009. https://doi.org/10.1002/ijc.32396.
[43] Marmolejo DH, Wong MYZ, Bajalica-Lagercrantz S, Tischkowitz M, Balmaña J. Overview of hereditary breast and ovarian cancer (HBOC) guidelines across Europe. Eur J Med Genet 2021;64:104350. https://doi.org/10.1016/j.ejmg.2021.104350.
[44] Heald B, Pirzadeh-Miller S, Ellsworth RE, Nielsen SM, Russell EM, et al. Cascade testing for hereditary cancer: comprehensive multigene panels identify unexpected actionable findings in relatives. J Natl Cancer Inst 2024;116:334–7. https://doi.org/10.1093/jnci/djad203.