Performance of breast cancer polygenic risk scores in 760 female *CHEK2* germline mutation carriers: A retrospective multicenter biomarker study

Julika Borde, MSc[1*], Corinna Ernst, MSc[1*], Barbara Wappenschmidt, PhD[1], Dieter Niederacher, PhD[2], Konstantin Weber-Lassalle, MSc[1], Gunnar Schmidt, PhD[3], Jan Hauke, PhD[1], Anne Quante, MD[4], Nana Weber-Lassalle, PhD[1], Judit Horváth, MD[5], Esther Pohl-Rescigno, PhD[1], Norbert Arnold, PhD[6], Andreas Rump, PhD[7], Andrea Gehrig, MD[8], Julia Hentschel, PhD[9], Ulrike Faust, PhD[10], Véronique Dutrannoy, Dipl.-Ing.[11], Alfons Meindl, MD[12], Maria Kuzyakova, MSc[13], Shan Wang-Gohrke, PhD[14], Bernhard H.F. Weber, PhD[15,16], Christian Sutter, PhD[17], Alexander E. Volk, MD[18], Olga Giannakopoulou, PhD[19,20], Andrew Lee, PhD[21], Christoph Engel, MD[22], Marjanka K. Schmidt, PhD[23], Antonis C. Antoniou, PhD[21], Rita K. Schmutzler, MD[1#], Karoline Kuchenbaecker, PhD[19,20#], Eric Hahnen, PhD[1#]

[*]These authors contributed equally as first authors.

[#]These authors contributed equally as last authors.

[1]Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany; [2]Department of Gynaecology and Obstetrics, University Hospital Duesseldorf, Heinrich-Heine University Duesseldorf, Duesseldorf, Germany; [3]Institute of Human Genetics, Hannover Medical School, Hannover, Germany; [4]Department of Gynecology and Obstetrics, Technical University Munich, University Hospital Rechts der Isar, Munich, Germany.[5]Institute for Human Genetics, University Hospital Muenster, Muenster, Germany; [6]Institute of Clinical Molecular Biology, Department of Gynaecology and Obstetrics, University Hospital of Schleswig-Holstein, Campus Kiel, Christian-Albrechts University Kiel, Kiel, Germany; [7]Institute for Clinical Genetics, Technische Universitaet Dresden, Dresden, Germany; [8]Institute of Human Genetics, Julius-Maximilians-Universität Würzburg, Würzburg, Germany; [9]Institute

of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Germany; [10]Institute of Medical Genetics and Applied Genomics, University Hospital Tuebingen, Tuebingen, Germany; [11] Institute of Medical and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany; [12]Department of Gynecology and Obstetrics, LMU Munich, University Hospital Munich, Munich, Germany; [13]Institute of Human Genetics, University Medical Center, Georg August University, Goettingen, Germany; [14]Department of Gynaecology and Obstetrics, University Hospital Ulm, Ulm, Germany; [15]Institute of Human Genetics, University of Regensburg, Regensburg, Germany; [16]Institute of Clinical Human Genetics, University Hospital Regensburg, Germany; [17]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany; [18]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [19]Division of Psychiatry, University College London, Maple House, London, UK;[20]UCL Genetics Institute, University College London, London, UK; [21]Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; [22]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany; [23]Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands.

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Corresponding author:

PD Dr. rer. nat. Eric Hahnen, MBA

Center for Hereditary Breast and Ovarian Cancer

University Hospital Cologne

Kerpener Str. 34, 50931 Cologne, Germany

Phone: +49 221 478-78929

Email: eric.hahnen@uk-koeln.de

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ABSTRACT

BACKGROUND: Genome-wide association studies (GWAS) suggest that the combined effects of breast cancer (BC)-associated single nucleotide polymorphisms (SNPs) can improve BC risk stratification using polygenic risk scores (PRSs). The performance of PRSs in GWAS-independent clinical cohorts is poorly studied in individuals carrying mutations in moderately penetrant BC predispositions genes such as *CHEK2*. **METHODS**: 760 female *CHEK2* mutation carriers were included; 561 women were affected with BC, of whom 74 developed metachronous contralateral BC (mCBC). For PRS calculations, two SNP sets covering 77 (SNP set 1, developed for BC risk stratification in women unselected for their *BRCA1/2* germline mutation status) and 88 (SNP set 2, developed for BC risk stratification in female *BRCA1/2* mutation carriers) BC-associated SNPs were used. **RESULTS:** Both SNP sets provided concordant PRS results at the individual level ($r=0.91$, $p<2.20\times10^{-16}$). Weighted cohort Cox regression analyses revealed significant associations of PRSs with the risk for first BC. For SNP set 1, a hazard ratio (HR) of 1.71 per standard deviation of the PRS was observed (95% confidence interval [CI]: 1.36 to 2.15, $p=3.87\times10^{-6}$). PRSs identify a subgroup of *CHEK2* mutation carriers with a predicted lifetime risk for first BC that exceeds the surveillance thresholds defined by international guidelines. Association of PRS with mCBC was examined via Cox regression analysis (SNP set 1 HR: 1.23, 95%CI: 0.86 to 1.78, p=0.26). **CONCLUSION:** PRSs may be used to personalize risk-adapted preventive measures for women with *CHEK2* mutations. Larger studies are required to assess the role of PRSs in mCBC predisposition.

INTRODUCTION

Personalized risk prediction is essential for optimized decision making in clinical management for women with a breast cancer (BC) family history[1-5]. Genome-wide association studies (GWAS) identified germline BC susceptibility loci, i.e. single nucleotide polymorphisms (SNPs), which were shown to modify BC risk in addition to germline mutations in established high-risk BC predisposition genes such as *BRCA1* and *BRCA2*[6-9]. The effects conferred by each of the BC-associated SNPs are low, but can be combined into polygenic risk scores (PRSs) which could achieve a significant degree of BC risk discrimination for women with or without a family history of BC and for women carrying pathogenic *BRCA1* or *BRCA2* mutations[10-12]. For female *BRCA1/BRCA2* mutation carriers, the effect of an 88 SNP-based PRS on BC risk was evaluated by Kuchenbaecker et al. in a GWAS dataset of 23,463 European mutation carriers[12]. For *BRCA1* mutation carriers with a PRS in the highest and lowest deciles, the calculated BC risks at the age of 60 years were 57% and 38%, respectively. For *BRCA2* mutation carriers, the risks to develop BC at the age of 60 was 40% in the highest decile and 28% in the lowest decile of the PRS distribution[12]. In a GWAS sample of 33,673 patients with BC and 33,381 control women of European descent, Mavaddat et al. demonstrated that a 77- SNP-based PRS stratifies BC risk in women unselected for their *BRCA1/2* germline mutation status[11]. A study focusing on 369 *CHEK2* c.1100delC germline mutation carriers (285 with BC; 84 without BC) and 33,624 non-carriers (17,640 cases; 15,984 controls) and the 77 BC-associated SNPs described by Mavaddat et al. revealed that the PRS was associated with an odds ratio (OR) of 1.59 (95% confidence interval [CI]: 1.21 to 2.09) per standard deviation for BC[13], similar to the OR estimated in non-carriers. Of note, both SNP sets described by Mavaddat et al. (subsequently referred to as SNP set 1) and Kuchenbaecker et al.(subsequently referred to as SNP set 2) do have 55 SNPs in common, and 13 SNPs unique to

SNP set 1 are in linkage disequilibrium to at least one out of 13 SNPs unique to SNP set 2, and vice versa. From the remaining 9 SNPs exclusive to SNP set 1, four were reported as specific for estrogen receptor (ER)-positive BC, and one for ER-negative BC, respectively, by Kuchenbaecker et al.[12].

In many European countries, the *CHEK2* gene is the third most frequently mutated BC risk gene[14], with c.1100delC (p.Thr367Metfs*15) being by far the most frequently observed pathogenic mutation. *CHEK2* germline mutations confer estimated lifetime risks (LTRs) for BC of approximately 20%[15] and a 3.5-fold increased risk for a second BC compared with non-carriers[16]. The performance of PRSs in GWAS-independent clinical cohorts is poorly studied for moderate penetrant risk genes and thus the clinical implementation of PRSs is pending for these individuals. Moreover, it remains elusive whether PRSs may predict the risk for secondary contralateral BC (CBC) in addition to that for unilateral BC. To address these issues, we analyzed a clinical cohort of women at increased risk for both uni- and contralateral BC because of carrying a pathogenic *CHEK2* germline variant.

PATIENTS AND METHODS

Study cohort of female *CHEK2* germline mutation carriers

Female individuals carrying monoallelic protein-truncating germline variants (PTVs) in the *CHEK2* gene (MIM +604373, transcript NM_007194.3) were eligible for this investigation. All individuals were identified through diagnostic germline testing in families recruited between January 1997 and June 2018 by 17 centers of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC). All families met the inclusion criteria of the GC-HBOC for germline testing of the respective index patient[17] (Supplementary Table 1). Demographic data, disease characteristics, family history, and medical history were documented in a central

registry at the Institute for Medical Informatics, Statistics, and Epidemiology (IMISE), University of Leipzig (Supplementary Table 2). Written informed consent was obtained from all individuals and ethical approval was granted by the Ethics Committee of the University of Cologne (07-048). All PTVs were classified as likely deleterious (class 4) or deleterious (class 5) based on the 5-tier variant classification system suggested by the International Agency for Research on Cancer (IARC) Unclassified Genetic Variants Working Group and in accordance to the regulations proposed by the international ENIGMA consortium[18] (Evidence-based Network for the Interpretation of Germline Mutant Alleles; https://enigmaconsortium.org; version 28, November 2019); class 4/5 PTVs were referred to as ´mutations´. For this investigation, we excluded *i) CHEK2* mutation carriers who were part of previous GWAS studies aimed at identifying SNPs associated with BC risk (iCOGs study[19]; OncoArray study[20]) and *ii) CHEK2* mutation carriers who also carried *BRCA1/2* mutations. This resulted in a sample of 769 female individuals.

Combined PRS-SNP genotyping by next generation sequencing

PRSs were computed based on a SNP set comprising of 77 SNPs developed using data on women unselected for germline mutation status (Mavaddat et al.[11]; SNP set 1) and a SNP set comprising of 88 SNPs developed for BC risk stratification for female *BRCA1/2* mutation carriers (Kuchenbaecker et al.[12]; SNP set 2). For SNP genotyping, we used a customized 48.48 amplicon-based target enrichment panel (Access Array®, Fluidigm, San Francisco, CA, USA). SNP panel design was assisted using the web-based D3 Assay Design tool (Fluidigm). Variants which could not be covered due to technical limitations, were replaced by adjacent SNPs in linkage disequilibrium (Supplementary Table 3). Subsequent parallel next generation sequencing (NGS) of the barcoded amplicons of 384 samples was performed by employing an Illumina NextSeq500 sequencing device (Ilumina, San Diego, CA, USA) (Supplementary

Methods). All DNA samples were centrally analyzed at the Center for Familial Breast and Ovarian Cancer, University Hospital Cologne, Germany.

Quality control of PRS-SNP genotyping results

For SNP set 1, 6 of 77 SNPs were excluded (rs1045485, rs7726159, rs12662670, rs13281615, rs8170, rs2363956; Supplementary Table 4). For SNP set 2, 6 of 88 SNPs were excluded due to SNP call rates below 0.95 (rs12048493, rs56963355, rs9257408, rs13281615, rs494406, rs146699004; Supplementary Table 4). SNP rs132390, located ~500 kb upstream of the *CHEK2* gene and considered in both SNP sets, was described to be associated with the *CHEK2* c.1100delC mutation[6, 21]. Consistently, the rs132390 minor allele frequency (MAF) in the subgroup of 557 *CHEK2* c.1100delC mutation carriers was significantly increased in comparison to 203 individuals carrying other *CHEK2* mutations (MAF = 0.35 vs MAF = 0.04, Fisher's exact test $p<2.2\times10^{-16}$). Thus, rs132390 unlikely defines an independent BC risk allele and was, therefore, excluded from the PRSs. In summary, these analyses resulted in an effective set of 70 SNPs for SNP set 1 and 81 SNPs for SNP set 2.

PRS computation

For each sample i, an individual PRS was derived via

$$PRS_i = \sum_{j=i}^{N} \beta_j g_{ij} \; with \; g \in \{0,1,2\},$$

where β is the per allele log odds ratio and g_{ij} is the number of effect alleles in person i for locus j. Missing genotypes were imputed to the average observed genotype in the sample (see Supplementary Methods). Values of $\overline{PRS}$, the theoretically expected mean PRSs with respect

to the final SNP sets, were derived as described by Kuchenbaecker et al.[12] using the European subset of 1000 Genomes data (Supplementary Methods).

Statistical analysis

Analyses were run employing the GenABEL v1.8 utilities[22] under R v3.6 and PLINK v1.9[23]. Quality controls including checks for duplicate samples and ethnicity outliers (Supplementary Methods), resulted in a curated data set comprising 760 *CHEK2* mutation carriers out of 578 families as input for the PRS computation.

PRSs for both SNP sets were standardized to have mean 0 and variance 1. To account for the non-random sampling of *CHEK2* mutation carriers with respect to their disease status, the association of standardized PRSs with BC risk was analyzed using a weighted cohort Cox regression[24] with time to first BC diagnosis as the outcome (Supplementary Methods). The weighted cohort approach aimed to correct the bias towards *CHEK2* mutation carriers affected with BC in the sample by assignment of adapted weights to individuals with and without BC per age group, such that the observed weighted incidence rate agrees with the expected population-based incidence rates of *CHEK2* mutation carriers[15, 25]. Observations were censored at age of first BC diagnosis, OC diagnosis, prophylactic mastectomy or age at last observation, whichever appeared at earliest. Analyses were adjusted for year of birth and counseling center of origin (Supplementary Methods).

Age-specific PRS analyses, i.e. examination of the proportional hazard assumption in age-stratified Cox regression models, were performed as described by Zhang et al.[26]. Absolute age-specific cumulative risks of developing BC at different percentiles of the standardized PRS, were calculated using age-specific hazard ratios (HRs) per standard deviation of the PRS as

described previously[12], based on UK incidences for women with *CHEK2* c.1100delC mutation using recently published relative risk estimates[15, 25].

CBC occurring within a year of the first BC diagnosis were defined as synchronous contralateral BC (sCBC) and those detected after one year as metachronous contralateral BC (mCBC).

A Cox regression from time from one year after BC diagnosis to mCBC as the outcome, was applied to individuals with BC but not with sCBC, to evaluate the association of PRSs and age at first BC diagnosis with mCBC (Supplementary Methods). To prevent a bias towards genetic testing due to mCBC, patients who entered the study after the occurrence of mCBC were excluded. Patients were censored at the age at mCBC diagnosis, OC diagnosis, prophylactic mastectomy of the healthy breast, or last observation.

RESULTS

The study sample consisted of 769 female *CHEK2* mutation carriers. After genotype quality control, 760 individuals were included, of whom 557 carried the c.1100delC mutation and 203 other PTVs (Supplementary Table 5). A total of 551 mutation carriers were diagnosed with BC (450 patients with unilateral BC only, with a mean age of 46.2 (range 23–78); 74 patients with mCBC: mean age at first BC diagnosis: 41.2 (range 25–64), at mCBC diagnosis: 49.5 (range 31–79); 27 patients with sCBC). 10 patients were diagnosed with BC and OC, 6 out of these were affected by OC prior to BC. 199 mutation carriers had not been diagnosed with BC. Key characteristics of the study sample are summarized in Supplementary Table 2.

The distribution of the raw PRSs for both SNP set 1 and SNP set 2 in the overall study sample are shown in Figure 1. For SNP set 1, the PRS ranged from -1.12 to 1.72 (mean 0.36) and for SNP set 2 from -1.44 to 1.73 (mean 0.20). For both SNP sets, the mean PRS in the overall study sample was significantly increased in comparison to the theoretically expected value

$\overline{PRS}$ (SNP set 1: p=2.95x10^{-14}, SNP set 2: p=1.49x10^{-5}; two-sided t-test; Figure 1A, B), reflecting the enrichment for BC and BC family history in the sample. Standardized PRSs from both SNP sets showed significant correlation at the individual level (Pearson's correlation coefficient r=0.91, p<2.20×10^{-16}; Figure 1C). Due to the high accordance between both SNP sets, results shown hereafter are restricted to SNP set 1, results for SNP set 2 are shown in the Supplementary Tables 7 to 9.

Weighted cohort Cox regression analysis revealed a significant association of the standardized PRS with BC risk (HR: 1.71, 95% CI: 1.36 to 2.15, p=3.87x10^{-6}) (Table 1). In addition, we tested whether the association between the c.1100delC mutation and BC differed from the association between other PTVs in *CHEK2* and BC. We also tested whether the PRS association was consistent across carriers of different *CHEK2* mutations by including an interaction term between the PRS and c.1100delC carrier status. The association with BC did not differ between c.1100delC and other PTVs (HR: 0.86, 95% CI: 0.52 to 1.40, p=0.53), nor did the association between PRS and BC differ significantly by c.1100delC carrier status (interaction HR 0.67, 95% CI: 0.42 to 1.08; p=0.1) (Table 1). Testing for the violation of the proportional hazards assumption pointed towards an interaction of the PRS with age (Table 2). The PRS showed stronger associations with first BC in younger age groups and associations were attenuated for ages >50 years (Table 2).

Age-specific HR estimates served as input for computation of absolute cumulative BC risks by PRS percentile (Figure 2). *CHEK2* mutation carriers at the 10th percentile of the PRS had a risk of 2% of developing BC by the age of 50 years and a 13% risk by the age of 80 years; mutation carriers at the 90th percentile of the PRS had an 11% BC risk by the age of 50 years and 33% by the age of 80 years. The high concordance of absolute cumulative BC risk predictions based on both SNP set 1 and SNP set 2 (Supplementary Figure 2), along with the high concordance

of standardized PRS calculations at individual level (Figure 1C), suggest a similar clinical utility for both SNP sets.

In addition to that for unilateral BC, we tested whether the PRS predicts the risk for CBC in a sample of 528 patients, including 34 individuals with mCBC. The mean interval between the first and the second BC was 8.3 years (range 1–30 years). Cox regression analyses with time from one year after first BC diagnosis to mCBC as the outcome, pointed towards an association of the PRS with mCBC, although a level of significance was reached only for the association of first BC age with mCBC risk (HR per standard deviation of the PRS: 1.23, 95% CI: 0.86 to 1.78, p=0.26; age at first BC diagnosis HR: 0.95, 95%CI: 0.86 to 1.78, p=0.26) (Table 3).

DISCUSSION

While the clinical management of women carrying pathogenic mutations in high-risk BC genes, such as *BRCA1* and *BRCA2* is well-established, the clinical management of women carrying mutations in moderately penetrant BC genes, such as *CHEK2*, is less standardized and may vary from country to country[27]. The clinical decision whether preventive measures are offered or not is mainly based on the estimated LTR and family history of cancer. We demonstrate that PRSs are suited to identify women with *CHEK2* mutations in a risk category that exceeds for example the surveillance thresholds for BC according to the UK National Health and Care Excellence (NICE) and US National Comprehensive Cancer Network (NCCN) guidelines. The NICE guidelines generally consider an annual mammography for women with an estimated LTR >30%, starting at the age of 30 years[28]. The NCCN recommends an annual mammography for patients with a LTR >20% starting earliest at the age of 30 years or at an age that is 10 years younger than the age of the earliest BC diagnosis in the family, whichever is later[29]. For *CHEK2* germline mutation carriers, the NCCN suggests an annual breast

screening by MRI starting at the age of 40 years, depending on the family history[30]. We demonstrate that the PRSs may be useful in identifying those women with risks exceeding the general surveillance thresholds of 20% or 30% LTR, respectively. In our study sample, on the basis of the PRS alone, approximately 10% of women with *CHEK2* mutations fall in a risk category that exceeds the surveillance thresholds for BC according to the NICE recommendations (112/760) and approximately 50% of these women fall in a risk category that exceeds the surveillance thresholds for BC according to NCCN guidelines (400/760).

Differences in the cumulative risk of mutation carriers within the highest decile of the PRS distribution were more than 5-fold increased compared to the lowest decile at the age of 50 years (>11% vs <2%), whereas this effect was attenuated by the age of 80 years (>33% vs <13%). The stronger association of the PRS in younger age groups are in line with the results published for *BRCA1/2* mutation carriers[12]. These data suggest that especially women younger than 50 years may benefit from a PRS-based risk prediction due to the higher levels of risk discrimination.

A study of women unselected for their germline mutation status demonstrated that a PRS based on 67 polymorphic loci associated with BC is associated with the development of CBC (WECARE study results)[31]. A recent study considering 99,969 women enrolled in the FinnGen study reported an association of PRS >90th percentile with mCBC risk in 5,979 Finnish individuals with BC, including 202 *CHEK2* c.1100delC mutation carriers, for a PRS including millions of loci[32]. We found no significant evidence of association between the PRS with mCBC in our study. However, our analysis was based on small number of mCBC cases and the estimates were associated with wide confidence intervals, and therefore effects of the PRSs cannot be excluded either.

In conclusion, PRSs have the potential to improve personal risk prediction accuracy for first and contralateral BC in *CHEK2* mutation carriers. Our GWAS-independent study was not restricted to the most common pathogenic *CHEK2* variant c.1100delC and we demonstrated that women with other PTVs in the *CHEK2* gene may benefit from the PRS-based BC risk prediction. The recently extended BOADICEA model[25] implemented in the CanRisk tool (www.canrisk.org) includes the effects of *CHEK2* mutations, family history, and the BC PRS. Our results support the use of such tools in a clinical setting for providing more personalized BC risks for *CHEK2* mutation carriers on the basis of the combined effects of family history and PRS.

This study has limitations. Our study sample consists of individuals who met the GC-HBOC inclusion criteria for germline testing. Therefore, a strong bias towards a familial BC background exists. Moreover, PRS analyses were restricted to individuals of European descent and our results may not be applicable to other populations.

Since *CHEK2* c.1100delC mutation carriers are more likely predisposed to ER-positive disease compared to non-carriers[13, 16], the question arises whether a ER-positive specific PRS may be more suitable for *CHEK2* mutation carriers. Here the overall PRS was utilized due to ER-negative mutation carriers in our cohort (52 out of 441 first BC cases with known receptor status were ER-negative (11.79%)) and a high correlation between the ER-positive BC and the overall BC PRS at the individual level (r=0.96 95% CI: 0.95 to 0.96).

Larger studies based on more recently defined 313 or 3,820 SNP-based PRS [10] will provide more precise estimates of the association of PRS with BC and mCBC.

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NOTES

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REFERENCES

1. Bleyer A, Welch HG. Effect of three decades of screening mammography on breast-cancer incidence. N Engl J Med 2012;367(21):1998-2005.

2. Cuzick J, Sestak I, Bonanni B*, et al.* Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. Lancet 2013;381(9880):1827-34.

3. Howell A, Astley S, Warwick J*, et al.* Prevention of breast cancer in the context of a national breast screening programme. J Intern Med 2012;271(4):321-30.

4. Pashayan N, Duffy SW, Chowdhury S*, et al.* Polygenic susceptibility to prostate and breast cancer: implications for personalised screening. Br J Cancer 2011;104(10):1656-63.

5. Burton H, Chowdhury S, Dent T*, et al.* Public health implications from COGS and potential for risk stratification and screening. Nat Genet 2013;45(4):349-51.

6. Michailidou K, Hall P, Gonzalez-Neira A*, et al.* Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45(4):353-61, 361e1-2.

7. Kuchenbaecker KB, Ramus SJ, Tyrer J*, et al.* Identification of six new susceptibility loci for invasive epithelial ovarian cancer. Nat Genet 2015;47(2):164-71.

8. Michailidou K, Beesley J, Lindstrom S*, et al.* Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet 2015;47(4):373-80.

9. Easton DF, Pooley KA, Dunning AM*, et al.* Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447(7148):1087-93.

10. Mavaddat N, Michailidou K, Dennis J*, et al.* Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. Am J Hum Genet 2019;104(1):21-34.

11. Mavaddat N, Pharoah PD, Michailidou K*, et al.* Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst 2015;107(5).

12. Kuchenbaecker KB, McGuffog L, Barrowdale D*, et al.* Evaluation of Polygenic Risk Scores for Breast and Ovarian Cancer Risk Prediction in BRCA1 and BRCA2 Mutation Carriers. J Natl Cancer Inst 2017;109(7):999-1009.

13. Muranen TA, Greco D, Blomqvist C*, et al.* Genetic modifiers of CHEK2*1100delC-associated breast cancer risk. Genet Med 2017;19(5):599-603.

14. Lilyquist J, Ruddy KJ, Vachon CM*, et al.* Common Genetic Variation and Breast Cancer Risk-Past, Present, and Future. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2018;27(4):380-394.

15. Schmidt MK, Hogervorst F, van Hien R*, et al.* Age- and Tumor Subtype-Specific Breast Cancer Risk Estimates for CHEK2*1100delC Carriers. J Clin Oncol 2016;34(23):2750-60.

16. Weischer M, Nordestgaard BG, Pharoah P*, et al.* CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. J Clin Oncol 2012;30(35):4308-16.

17. Kast K, Rhiem K, Wappenschmidt B*, et al.* Prevalence of BRCA1/2 germline mutations in 21 401 families with breast and ovarian cancer. J Med Genet 2016;53(7):465-71.

18. ENIGMA. *Evidence-based Network for the interpretation of Germline Mutant Allels*. https://enigmaconsortium.org/.

19. Sakoda LC, Jorgenson E, Witte JS. Turning of COGS moves forward findings for hormonally mediated cancers. Nat Genet 2013;45(4):345-8.

20. Amos CI, Dennis J, Wang Z*, et al.* The OncoArray Consortium: A Network for Understanding the Genetic Architecture of Common Cancers. Cancer Epidemiol Biomarkers Prev 2017;26(1):126-135.

21. Bahcall O. Shared susceptibility loci for breast, prostate and ovarian cancers. Nature Genetics 2019; 10.1038/ngicogs.2.

22. Aulchenko YS, Ripke S, Isaacs A*, et al.* GenABEL: an R library for genome-wide association analysis. Bioinformatics 2007;23(10):1294-6.

23. Chang CC, Chow CC, Tellier LC*, et al.* Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015;4:7.

24. Antoniou AC, Goldgar DE, Andrieu N*, et al.* A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. Genet Epidemiol 2005;29(1):1-11.

25. Lee A, Mavaddat N, Wilcox AN*, et al.* BOADICEA: a comprehensive breast cancer risk prediction modelincorporating genetic and nongenetic risk factors. Genetics in Medicine 2019;21(8):1708-1718.

26. Zhang Z, Reinikainen J, Adeleke KA*, et al.* Time-varying covariates and coefficients in Cox regression models. Annals of translational medicine 2018;6(7):121-121.

27. Kleiblova P, Stolarova L, Krizova K*, et al.* Identification of deleterious germline CHEK2 mutations and their association with breast and ovarian cancer. Int J Cancer 2019;145(7):1782-1797.

28. National Collaborating Centre for C. National Institute for Health and Clinical Excellence: Guidance. In. *Familial Breast Cancer: Classification and Care of People at Risk of Familial Breast Cancer and Management of Breast Cancer and Related Risks in People with a Family History of Breast Cancer*. Cardiff (UK): National Collaborating Centre for Cancer (UK)

29. Network NCC. NCCN clinical practice guidelines in oncology: breast cancer screening and diagnosis, Version 1. In; 2019, May 17.

30. Oncology. NCPGi. *Genetic/Familial High-Risk Assessment: Breast and Ovarian*. nccn.org.

31. Robson ME, Reiner AS, Brooks JD*, et al.* Association of Common Genetic Variants With Contralateral Breast Cancer Risk in the WECARE Study. JNCI: Journal of the National Cancer Institute 2017;109(10).

32. Mars N, Widen E, Kerminen S*, et al.* Polygenic risk, susceptibility genes, and breast cancer over the life course. medRxiv 2020; 10.1101/2020.04.17.20069229:2020.04.17.20069229.

Table 1: Hazard ratio per standard deviation of the standardized PRS (HR), 95% confidence intervals (CI) and p values (p, two-sided Wald test) for the association of PRS with BC risk in 760 female *CHEK2* mutation carriers, with and without inclusion of an interaction term for the PRS with c.1100delC carrier status.

	SNP set 1		
	HR	**CI**	**p**
Weighted Cox regression			
PRS	1.71	1.36 to 2.15	3.87×10^{-6}
Weighted Cox regression with PRS x c.1100delC interaction			
PRS	2.29	1.56 to 3.38	2.70×10^{-5}
c.1100delC	0.86	0.53 to 1.41	0.56
PRSxc.1100delC interaction term	0.67	0.42 to 1.08	0.10

Table 2: Age-specific hazard ratios per standard deviation of the standardized PRS (HR), 95% confidence intervals (CI) and p values (p, two-sided Wald test) for the association with of PRS with BC risk, and two-sided p values for testing for the proportional hazards assumption of the Cox model stratified by age groups (p value for PRS: PRS, p value for global test: GLOBAL).

			SNP set 1		
Age category	**No. at risk**	**No. of events**	**HR**	**CI**	**p**
≤ 40	760	163	1.43	1.04 to 1.97	0.03
41–50	503	254	2.32	1.69 to 3.20	2.62×10^{-7}
51–60	204	96	1.59	1.07 to 2.35	0.02
> 60	71	42	1.34	0.78 to 2.30	0.29
PRS					0.15
GLOBAL					9.50×10^{-91}

Table 3: Hazard ratios per standard deviation of the standardized (HR), 95% confidence intervals (CI) and p values (p, two-sided Wald test) for a Cox model with time from one year after first BC diagnosis to mCBC as the outcome, including 528 *CHEK2* mutation carriers with BC, but not sCBC. The standardized PRS do not show significant association with mCBC. A significant negative association with age at first diagnosis was observed.

	SNP set 1		
	HR	**CI**	**p**
PRS	1.23	0.86 to 1.78	0.26
Age at first BC diagnosis (years)	0.95	0.92 to 0.98	3.67×10^{-4}

Figure 1: (A, B) Empirical distributions of the PRS for SNP set 1 (A) and SNP Set 2 (B) in the overall cohort of 760 *CHEK2* mutation carriers. Values of theoretically expected mean $\overline{PRS}$, are indicated with vertical lines. (C) Standardized PRS values per individual for SNP set 1 and SNP set 2. Abbreviations: PRS, polygenic risk score; SNP, single nucleotide polymorphism.

Figure 2: Predicted cumulative BC risks by percentile of the PRSs in *CHEK2* mutation carriers.