# Genetic modifiers of CHEK2*1100delC-associated breast cancer risk

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# Genetic modifiers of CHEK2*1100delC associated breast cancer risk

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## Abstract

**Purpose—***CHEK2**1100delC is a founder variant in European populations conferring a 2–3 fold increased risk of breast cancer (BC). Epidemiologic and family studies have suggested that the risk associated with *CHEK2**1100delC is modified by other genetic factors in a multiplicative fashion. We have investigated this empirically using data from the Breast Cancer Association Consortium (BCAC).

**Methods—**With genotype data of 39,139 (624 1100delC carriers) BC patients and 40,063 (224) healthy controls from 32 BCAC studies, we analyzed the combined risk effects of *CHEK2**1100delC and 77 common variants in terms of a polygenic risk score (PRS) and pairwise interaction.

**Results—**The PRS conferred an odds ratio (OR) of 1.59 [95% CI 1.21–2.09] per standard deviation for BC for *CHEK2**1100delC carriers and 1.58 [1.55–1.62] for non-carriers. No evidence for deviation from the multiplicative model was found. The OR for the highest quintile of the PRS was 2.03 [0.86–4.78] for *CHEK2**1100delC carriers placing them to the high risk category according to UK NICE guidelines. OR for the lowest quintile was 0.52 [0.16–1.74], indicating life-time risk close to population average.

**Conclusion—**Our results confirm the multiplicative nature of risk effects conferred by *CHEK2**1100delC and the common susceptibility variants. Furthermore, the PRS could identify the carriers at a high life-time risk for clinical actions.

### Keywords

Breast cancer; *CHEK2**1100delC; Polygenic risk score (PRS); common variants; Breast Cancer Association Consortium (BCAC)



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## INTRODUCTION

The protein truncating mutation *CHEK2**1100delC (checkpoint kinase 2) is a moderate penetrance breast cancer risk variant with relative risk estimate of 2–3 fold.[1, 2] However, several studies have shown that the cumulative life-time risk of breast cancer in *CHEK2**1100delC carriers is markedly higher in women with a family history than without,[3–5] and that *CHEK2**1100delC carriers have a higher probability of developing bilateral breast cancer.[6] These observations are quantitatively consistent with a simple polygenic model suggesting that *CHEK2**1100delC combines multiplicatively with other genetic loci. However, this has not yet been established empirically.

Genome wide association studies have identified common genetic variants that are associated with increased risk of breast cancer. A polygenic risk score (PRS), based on 77 low penetrance variants has been estimated to explain approximately 12–14% of the excess familial risk and shown to identify individuals at high risk at the population level.[7, 8] Some of these variants predominantly predispose to either estrogen receptor positive (ER+) or estrogen receptor negative (ER−) disease, which represent the two main etiological subclasses of breast cancer.[9] *CHEK2**1100delC carriers are more strongly predisposed to ER+ disease: about 90% of carrier tumors are ER+ in comparison to 77–78% of non-carrier tumours.[10]

Here, we investigate the synergistic risk effects attributable to *CHEK2**1100delC and the common breast cancer susceptibility variants both individually and summarized in terms of the PRS.[7, 8]

## PATIENTS AND METHODS

### Study participants

Female invasive breast cancer patients and healthy controls of European ancestry were included from studies participating in the Breast Cancer Association Consortium (BCAC) (Table S1). Data from a study were included if the study provided genotype data of the common variants from at least one breast cancer patient carrying the 1100delC variant. This selection yielded data from 32 studies and a total of 79,202 study subjects, including 848 *CHEK2**1100delC carriers (Table S2) for pairwise interaction analyses. Complete quality controlled[7, 10] genotype data for all common variants and *CHEK2**1100delC were available from 33,624 study subjects (369 *CHEK2**1100delC carriers, Table S2). This data were used in the analyses involving the PRS.

All participating studies were approved by their institutional review committees. Each study followed national guidelines for participant inclusion and informed consent procedures.

### Genotyping

All variants except *CHEK2**1100delC were genotyped centrally using a custom Illumina iSelect genotyping array (iCOGS, Illumina, Inc. San Diego, CA, USA) as part of the COGS consortium studies as described earlier.[7, 8] *CHEK2**1100delC was primarily genotyped using a custom made TaqMan assay (Applied Biosystems, Foster City, CA, USA), with a

small minority being genotyped using iPLEX.[10] In addition to the 38,549 study subjects genotyped using the iCOGS array, 40,653 BCAC study subjects were genotyped for up to 25 of the common risk variants and these data were used in the pairwise interaction analysis (Table S2, Table S3). These samples were genotyped by independent studies following BCAC genotyping standards as described previously.[11, 12]

## Statistical analyses

Statistical analyses were performed using Stata SE 10 (StataCorp, College Station, Texas, USA) and R version 2.15.2.[13] For the common variants a log-additive model was assumed; i.e. the risk was analyzed in terms of the number of disease-associated alleles [0,1,2] carried. *CHEK2**1100delC was assumed to follow a dominant inheritance model as the number of rare homozygotes was small (n=19). All analyses were adjusted for study and seven principal components defined on the basis of the genome-wide data from the iCOGS project as described previously.[7] All reported tests were two-sided.

## Polygenic risk score

In order to investigate the combined effects of common variants and *CHEK2**1100delC, a polygenic risk score (PRS) based on the main effects of the common variants was calculated using the formula:

$$\sum_{i=1}^{n} a_i \log_2 OR_i$$

where n is the number of loci included in the model, a is the number of susceptibility alleles in locus i and OR is the per allele odds ratio for breast cancer, estimated separately for each variant in the whole data set (Table S4a, column “All”). Results using a PRS based on previously reported ORs[7, 8] were essentially identical (data not shown). The PRS was approximately normally distributed in all study subgroups, and was standardized by mean and standard deviation of the PRS among the healthy individuals.[8] For pairs of linked variants with r2>0.75, we included in the PRS only the lead variant (rs2981579, not rs2981582; rs12662670, not rs3757318; rs554219, not rs614367). We excluded two variants (rs78540526 and rs75915166) included in the PRS of Mavaddat et al.[8], which were not genotyped on the iCOGS array, as well as rs17879961, the *CHEK2* missense variant I157T, because the number of study subjects carrying both 1100delC and I157T was very low (n=5). Thus, the resulting PRS included 74 variants. The interaction between PRS and *CHEK2**1100delC was assessed by comparing nested logistic regression models: a model including the PRS and 1100delC genotype and a model supplemented with an interaction term, coded as the product of the PRS and 1100delC. In analyses of the PRS and positive family history of breast cancer, positive family history was defined as at least one first degree relative with breast cancer.

The cumulative life-time breast cancer risk of *CHEK2**1100delC carriers in different PRS-percentiles was derived assuming an average life-time risk of 22% for CHEK2*1100delC carriers[14] and previously published relative risk estimates associated with the PRS.[8]

### Pairwise interaction analyses

We tested for pairwise interaction between each common variant and *CHEK2**1100delC as described above for the interaction between the PRS and 1100delC. P-values were corrected for 77 parallel tests using the Benjamini-Hochberg method.[15] The OR for breast cancer was estimated separately for each of the common variants for the whole dataset and for the subgroup of 1100delC carriers. These analyses were also performed separately on a subgroup of breast cancer patients with ER+ disease, because 1100delC is associated with ER+ breast cancer.[10] We tested for heterogeneity in the ORs among different BCAC studies by including an interaction term between variant and the study, separately for each variant. No significant heterogeneity was found for any variant (data not shown). Statistical power was estimated as previously suggested for risk interaction analyses.[16]

## RESULTS

We analyzed the combined effects of *CHEK2**1100delC and common low penetrance breast cancer risk variants using data from the international Breast Cancer Association Consortium (Table S2). The PRS summarizing the individual effects of 74 common variants was strongly associated with breast cancer risk among *CHEK2**1100delC carriers (OR per unit standard deviation 1.59 [1.21–2.09], P=0.0008) and the OR was similar to that in non-carriers (1.58 [1.55–1.62], $P_{interaction}$ 0.93). ORs for the highest and lowest quintiles of the PRS distribution were 2.03 [0.86–4.78] and 0.52 [0.16–1.74] for *CHEK2**1100delC carriers, respectively, when compared to the middle quintile (Table 1). Both estimates were similar to those among non-carriers.

The OR associated with *CHEK2**1100delC in the analysis data set 2.99 [2.32–3.85] was attenuated, when the model was adjusted for positive family history of breast cancer. The OR associated with the PRS was also slightly attenuated (Table 2). No significant interaction between risk effects associated with 1100delC, PRS and positive family history was found. However, in a case-only analysis there was a significant association between the PRS and family history of breast cancer, among both *CHEK2**1100delC carriers (OR 1.29 [1.01–1.65], P=0.04) and non-carriers (OR 1.17 [1.12–1.21], P=4E-16) (Figure S1).

When altogether 77 common variants were considered individually, we found nominally significant interactions between five variants and *CHEK2**1100delC for overall breast cancer (rs11249433, rs11780156, rs204247, rs2981582 and rs704010; Table S4a). Two of these represented synergistic (more than multiplicative) and three antagonistic interactions (the estimated effect in 1100delC carriers being in the opposite direction to that in non-carriers). However, none of the interactions were significant after correction for multiple testing. Nine variants showed a nominally significant interaction for ER-positive breast cancer (Table S4b).

## DISCUSSION

Our analyses on the synergistic effects of *CHEK2**1100delC and 77 common low penetrance variants on breast cancer risk give strong support to the predicted multiplicative polygenic model.[8, 17, 18] While this has previously been shown for combinations of low

penetrance variants,[8] and for variants in combination with BRCA1 and BRCA2 mutations,[19] this is the first direct demonstration for a “moderate” risk gene and has important implications for risk prediction. The PRS was a significant risk factor for *CHEK2**1100delC carriers, and the estimated OR per unit standard deviation was very similar in *CHEK2**1100delC carriers and in non-carriers, consistent with the hypothesis that the common susceptibility variants combine with the rare *CHEK2**1100delC variant in an approximately multiplicative fashion. Similarly, the PRS risk estimates for the highest and lowest quintiles did not differ between the *CHEK2**1100delC carriers and non-carriers. These two estimates in the *CHEK2**1100delC carriers alone did not reach statistical significance (Table 1), possibly reflecting limited statistical power due to the relatively low number of healthy variant carriers (Table S2). However, this is the largest study genotyped for *CHEK2**1100delC and these common variants, and even though some of the point estimates are not significant, they are consistent with the previous reports. Most importantly, we did not find evidence for deviation from the multiplicative model, suggesting that the PRS could be used in risk stratification of 1100delC carriers in a similar manner to non-carriers.

The unadjusted OR for the *CHEK2**110delC variants (Table 2) was higher in our analysis data set than in previous reports.[2, 14] Adjusting for positive family history markedly attenuated the *CHEK2**1100delC associated OR, suggestive of some oversampling of familial cases. The PRS OR was also slightly attenuated after the adjustment. However, *CHEK2**1100delC, PRS and family history remained significant risk factors in the combined model (Table 2) suggesting that the common variants together explain part of the excess familial risk as previously suggested,[17] but that the PRS has predictive value also in breast cancer families segregating *CHEK2**1100delC.

Recently, a large study estimating the risk associated with *CHEK2**1100delC in relation to age, tumor subtype and family history reported the cumulative life-time risk for 1100delC carriers to be about 22%.[14] Assuming that the relative effect of the PRS is the same in carriers and non-carriers (OR higher than 1.48 [1.39–1.57] or lower than 0.65 [0.60–0.70] for percentiles above 80% or lower than 20%, respectively),[8] 20% of the 1100delC carriers with highest PRS would have life-time risk higher than 32.6% [30.6%–34.5%] exceeding the threshold for the high-risk category (>30%) according to the UK NICE guidelines for familial breast cancer.[20] Similarly, for the 20% of 1100delC carriers with lowest PRS, the life-time risk would be lower than 14.3% [13.2%–15.4%], i.e. close to the average population risk. These observations imply that, if *CHEK2**1100delC is to be used in risk prediction, it can be made more effective by including the PRS, representing the risk modifying effects of common variants, in the prediction.

*CHEK2**1100delC carrier cancers do not represent a phenotypically distinct subgroup of breast carcinomas. Instead, the phenotypic diversity of *CHEK2**1100delC associated cancers resembles that of breast tumors in general.[10] Thus, it was not surprising that the relative risks conferred by the common variants were similar for the *CHEK2**1100delC carriers and for non-carriers, and no significant pairwise interaction was found. We estimated that we had sufficient statistical power (80%, at $P<0.05$) to detect a pairwise interaction between *CHEK2**1100delC and any of the common variants, if the interaction

OR was 2.5 or greater, but not enough power to detect interactions comparable in magnitude to the risk effects associated with the low penetrance variants (OR 1.1–1.5). Thus, it remains possible that more modest departures from a multiplicative model may exist. If so, however, much larger case-control studies, perhaps combined with pedigree analyses, will be required to detect them.

In conclusion, our analyses confirm the predicted multiplicative relationship between *CHEK2**1100delC and the common low penetrance variants. Hence, the PRS could be similarly applied for risk prediction for the variant carriers as for the general population. Most importantly, the PRS could help identifying the high risk group of the *CHEK2**1100delC carriers, who would best benefit from clinical intervention.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

Breast cancer risk associated with the polygenic risk score (PRS) for non-carriers and the carriers of CHEK2*1100delC.

| | Non-carriers | | CHEK2*1100delC carriers | |
|---|---|---|---|---|
| | **OR [95% CI]** | **P** | **OR [95% CI]** | **P** |
| **PRS**[a] | 1.58 [1.55 – 1.62] | <1.0E-10 | 1.59 [1.21 – 2.09][b] | 0.0008 |
| **Percentile of PRS, %** | | | | |
| **< 20** | 0.52 [0.48 – 0.56] | <1.0E-10 | 0.52 [0.16 – 1.74] | 0.29 |
| **20–40** | 0.78 [0.72 – 0.84] | 2E-11 | 0.72 [0.28 – 1.88] | 0.51 |
| **40–60** | referent | | referent | |
| **60–80** | 1.25 [1.16 – 1.34] | 8E-10 | 0.93 [0.39 – 2.25] | 0.88 |
| **> 80** | 1.92 [1.80 – 2.06] | <1.0E-10 | 2.03 [0.86 – 4.78] | 0.11 |

[a]Odds ratio (OR) was estimated per unit standard deviation of the PRS.

[b]P-value for pairwise interaction between CHEK2*1100delC and PRS: 0.93.

## Table 2

Relative breast cancer risk associated with CHEK2*1100delC, PRS and positive family history of breast cancer in the analysis data set.

| Risk model | Parameters | OR [95% CI] | P |
|---|---|---|---|
| **BC ~ 1100delC + PRS** | 1100delC | 2.99 [2.32 – 3.85] | <1.0E-10 |
| | PRS | 1.58 [1.55 – 1.62] | <1.0E-10 |
| **BC ~ 1100delC + PRS + family history** | 1100delC | 2.42 [1.71 – 3.47] | 9.4E-7 |
| | PRS | 1.55 [1.50 – 1.60] | <1.0E-10 |
| | family history[a] | 2.73 [2.48 – 3.47] | <1.0E-10 |

[a]No significant interaction between positive family history of breast cancer and either *CHEK2**1100delC or PRS was found.