The path to individualised breast cancer screening

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CHAPTER 4

Validation of the BOADICEA model and a 313-variant polygenic risk score for breast cancer risk prediction in a Dutch prospective cohort

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Abstract

Purpose: We evaluated the performance of the recently extended Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA version 5) in a Dutch prospective cohort, using a Polygenic Risk Score based on 313 breast cancer-associated variants (PRS_{313}), and other, non-genetic risk factors.

Methods: Since 1989, 6,522 women without breast cancer (BC) aged 45 or older of European descent were included in the Rotterdam Study. The PRS_{313} was calculated per 1 standard deviation (SD) in controls from the Breast Cancer Association Consortium (BCAC). Cox regression analysis was performed to estimate the association between the PRS_{313} and incident BC risk. Cumulative 10-year risks were calculated with BOADICEA including different sets of variables (age, risk factors and PRS_{313}). C-statistics were used to evaluate discriminative ability.

Results: In total, 320 women developed BC. The PRS_{313} was significantly associated with BC (HR per SD of 1.56, 95%CI [1.40-1.73]). Using 10-year risk estimates including age and the PRS_{313}, other risk factors improved the discriminatory ability of the BOADICEA model marginally, from a C-statistic of 0.636 to 0.653.

Conclusion: The effect-size of the PRS_{313} is highly reproducible in the Dutch population. Our results validate the BOADICEA v5 model for BC risk assessment in the Dutch general population.

Introduction

Breast cancer is the most common cancer among women in Europe[1]. In the Netherlands, the average lifetime risk for developing invasive breast cancer is 13.6% for each woman, with the incidence peaking between 60-70 years of age[2]. Mammographic screening has decreased breast cancer mortality at the cost of detecting more disease that otherwise would not have become clinically apparent[3, 4]. Based on the UK guidelines, for every 10,000 women invited for screening at age 50 for the following 20 years, 43 deaths would be prevented, while 129 breast cancers would be overdiagnosed[5]. Furthermore, breast cancer screening inevitably yields false positives which can lead to anxiety[6]. Improvement of this benefit-to-harm ratio could be achieved by targeting women who benefit the most from screening, in particular those in the highest risk categories, while reducing screening for those in the lowest risk categories, potentially reducing overdiagnosis and costs while maintaining a reduced breast cancer death rate and improved life quality[7].

Many risk prediction algorithms have been developed to quantify the combined effect of various risk factors to predict the risk of developing breast cancer[8, 9]. The recently extended Breast and Ovarian analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) calculates cumulative risk of developing breast cancer based on family history, mammographic density, several lifestyle/hormonal and genetic risk factors[10]. BOADICEA includes the rare high to moderate risk pathogenic variants in breast cancer genes *BRCA1, BRCA2, PALB2, CHEK2* and *ATM,* and a Polygenic Risk Score (PRS) based on 313 breast cancer-associated variants (PRS_{313}). In 10 prospective studies, this PRS showed an association with breast cancer with an OR of 1.61 per standard deviation of the PRS distribution[11], and an area under receiver-operator curve of 0.630. It has been shown that the greatest breast cancer risk stratification in the general population and in women with a family history of breast cancer can be obtained by using the combined effects of the PRS and lifestyle/hormonal risk factors in the BOADICEA model[10].

Currently, breast cancer screening in the Dutch population is age-based[12]. Women start at age 50 years with biannual mammograms until the age of 75. Before considering risk-stratified approaches based on BOADICEA, it is important to assess its clinical validity in the Dutch population. In this study we validated the association between the PRS_{313} and breast cancer in a Dutch prospective cohort, its effect on predicting *in situ* breast cancer, and explore the discriminative ability of an individualised 10-year breast cancer risk score based on the PRS_{313} and several known risk factors using the BOADICEA version 5 model. We also assessed how a risk-based approach of population-based screening could have impacted breast cancer detection rates in our study cohort.

Materials and Methods

Study cohort

The Rotterdam Study (RS) is a prospective population-based cohort study of elderly Dutch individuals living in the Ommoord district of Rotterdam in the Netherlands[13]. Briefly, in the year 1989, individuals aged 55 or older were recruited into the RS-I cohort, which was extended in 2000 under similar criteria (RS-II-cohort) and in 2006 by the inclusion of individuals with an age between 45 and 55 (RS-III cohort). The overall response rate was 72%. In 2008 the Rotterdam Study comprised 14,926 subjects aged 45 years or older, including 8,823 women. For our study, we included all 6,670 women for whom genotype data were available. Genotyping was not performed for the excluded 2,153 women because of a low-quality DNA sample or because they declined blood-donation for DNA at study-entry.

Ethics statement

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the Dutch Ministry of Health, Welfare and Sports. All participants provided written informed consent to participate in the study and to have their medical information obtained from treating physicians.

Phenotype data

Diagnoses of cancer were collected for all individuals up to January 2014 and were based on medical records of general practitioners (including hospital discharge letters) and through linkage with Dutch Hospital Data, Netherlands Comprehensive Cancer Organisation, and histology and cytopathology registries in the region[13]. In total, 468 women had a breast cancer (invasive or *in situ*) diagnosis of whom 148 had been diagnosed prior to entry into the Rotterdam Study, and were excluded from further analyses. All participants were interviewed at home at inclusion, underwent extensive examinations every ~5 years in the Rotterdam Study research facility and received follow-up questionnaires (Figure S1), as described elsewhere[13]. Basic characteristics such as date of birth, vital status and age at inclusion were known for all participants. For most participants, information of breast cancer risk factors was available (Table S1, Total cohort), but family history of breast cancer and mammographic density were lacking. For the analyses, we used only information from the first questionnaire (Figure S1: RS-I-1, RS-II-1, RS-III-1) at the time of inclusion in the Rotterdam Study for variables that could vary over time, e.g. weight and alcohol use. Age at menopause was only included if menopause occurred before enrolment into the Rotterdam Study (Table S1, Subcohort).

Genotype data

Genotyping was performed with the Illumina 550K (RS-I and RS-II cohorts) and 610K (RS-III cohorts) arrays[13]. Standard quality control was completed, including selection on European ancestry, and imputation was performed using the Haplotype Reference Consortium (HRC) 1.1 and 1000G phase 3 reference panels[14, 15]. Of the 313 variants used to calculate the Polygenic Risk Score, 28 were directly genotyped by the arrays. Two variants were imputed with a quality below 0.3 and the remaining 283 variants were imputed with an average imputation quality of 0.95 (Table S2).

Polygenic Risk Score calculation

The following formula was used to calculate the PRS based on 313 variants:

$$PRS_j = \sum_{i=1}^{313} n_{ij} \ln (OR_i)$$

where n_{ij} is the number of risk alleles (0, 1 or 2) for variant i carried by individual j and OR_i is the per-allele odds ratio (OR) for breast cancer associated with variant i. The ORs were obtained from the Breast Cancer Association Consortium (BCAC) study[11] (Table S2). As the Estrogen Receptor (ER) status of the breast tumours was not available, only the overall breast cancer PRS was calculated. The PRS_{313} was standardised to the mean in all included women from the Rotterdam Study who did not develop incident breast cancer. To allow for direct comparison of PRS performance between both studies, the Standard Deviation (SD) of the population controls included in the validation-set from the BCAC study[11] was used, which was 0.609. For the calculations with BOADICEA version V, the PRS_{313} was standardised to the mean and SD from the population controls included in the total dataset from the BCAC study[11], which was -0.424 and 0.603 respectively.

Cumulative risk score calculation

Cumulative 10-year breast cancer risks were calculated with BOADICEA version V[10], starting at the age of inclusion in the Rotterdam Study, and using the birth-cohort incidence rates in combination with four different sets of variables, i.e., (i) age, (ii) age and PRS_{313}, (iii) age and risk factors, (iv) age, PRS_{313}, and risk factors. Risk factors included are age at menarche, age at menopause, number of children, age at first live birth, use of oral contraception, use of hormone replacement therapy, Body Mass Index (BMI), height, and alcohol use. For the variables that could vary over time, we used fixed variables. As BOADICEA ignores any risk factors for which the value is missing[10], no imputation was performed, and missing variables were kept missing.

Because BOADICEA calculates cumulative breast cancer risks up to age 80, 10-year breast cancer risks were only calculated for 4,377 women with an age of inclusion up to the age of 70 years. Women were considered affected if they developed breast cancer (invasive or *in situ*) within 10 years after inclusion in the Rotterdam Study.

Statistical analyses

Cumulative incidences were calculated using the Kaplan Meier method.

Association analyses

To estimate the association between the PRS_{313} and breast cancer risk in the Rotterdam Study cohort, Cox-regression analyses were performed. Relatedness among individuals of the same family was accounted for by correcting standard errors using a sandwich estimator. All models were adjusted by the age at inclusion in the Rotterdam Study. Incident breast cancer, *in situ* or invasive, was the event of interest. The time at risk was defined as the time elapsed between the inclusion date and the date of occurrence of the event of interest or right censoring. Right censoring could be due to (i) end of follow-up in January 2014 or (ii) death. The proportional hazard assumption for the model was tested. Sensitivity analyses were performed for (i) invasive breast cancer only by censoring the *in situ* breast cancer cases, (ii) *in situ* breast cancer only by censoring the invasive breast cancer cases, (iii) by censoring at the age of diagnosis of another type of cancer and (iv) by stratifying on Rotterdam Study cohort. To define the association between the PRS_{313} and other tumours than breast cancer, similar Cox-regression analysis was performed by censoring the breast cancer cases if they did not develop another tumour before the breast cancer diagnosis.

To investigate if the linearity assumption for the effect of PRS_{313} holds, we ran the model considering the categorical covariate given by the percentile groups of the PRS_{313} (0-10%; 10-20%; 20-40%; reference 40-60%; 60-80%; 80-90%; 90-100%) based on the distribution in the unaffected women in this cohort. The discrimination ability of the PRS_{313} in our sample was evaluated using the C-statistic[16], by groups based on quantiles of the age of inclusion in the Rotterdam Study (i.e. age <60, 60-70 and ≥70 years). Differences in the C-statistics were tested by computing bootstrap confidence intervals for the differences among groups.

Age-varying effect

The possible time-varying association of the PRS_{313} with breast cancer was investigated using age as time scale and considering three age dependent coefficients in the Cox model, corresponding to three different age intervals: (i) younger than 50 years, (ii) between 50 and 75 years old and (iii) above 75 years old. These cut-offs were chosen

based on their clinical relevance since women between 50 and 75 years are eligible for population screening according to the Dutch guideline[12].

Clinical validity of BOADICEA v5

To validate the BOADICEA 10-year cumulative risk scores, model calibration and discrimination ability in our sample were assessed. Calibration was investigated by comparing overall observed versus expected cumulative risks and by visually inspecting the calibration plots based on risk deciles. Because of the presence of right censoring, empirical risks at 10 years were estimated using the Kaplan-Meier method. As in the association analyses, discrimination was evaluated using C-statistics.

Statistical significance was defined as a two-sided p-value of <0.05. All analyses were performed with R version 3.5.3.[17]

Results

We included 6,522 women in the main analyses with an average age at study-entry of 66 years. Of these, 320 developed either invasive or *in situ* breast cancer during follow-up and 744 developed another type of tumour; the overlap between these two groups was 16, all of whom developed another type of tumour first (Table S3). The median follow-up calculated with the reverse Kaplan-Meier method was 12.40 years, with a minimum and maximum follow up of 0.03 and 24.43 years. Cohort characteristics are shown in Table S1. The average PRS_{313} in groups of affected (i.e. invasive, *in situ*, and a second breast tumour) and unaffected women (including women who developed another tumour than breast cancer) are shown in Figure S2 and Table S4.

Breast cancer cumulative incidence

The cumulative incidence of breast cancer in the total cohort was on average 4.2%, 95%CI [3.7%-4.8%] and 7.3%, 95%CI [6.4%-8.2%] 10 and 20 years after inclusion respectively. Stratified by quintiles of the PRS_{313}, after 20 years of follow-up, the incidence in the highest quintile was 10.8%, 95%CI [8.5%-13.1%] and 4.4%, 95%CI [2.8%-6.0%] for the lowest quintile (Figure S3).

Association analyses

A significant association was found between the PRS_{313} and incident breast cancer with an HR per SD of 1.56, 95%CI [1.40-1.74], $p=2.47 \times 10^{-15}$ (Table 1). There was no evidence of violation of the proportional hazard assumption (p-value=0.716), indicating that the HR remained constant over time. The discriminative ability of the PRS_{313}, as measured by the C-statistic, was 0.632, 95%CI [0.58-0.69], 0.673, 95%CI [0.61-0.73], and 0.562, 95%CI

[0.48-0.62] for women included before age 60, between age 60 and 70, and above age 70 respectively (Table 1).

Sensitivity analyses for (i) invasive breast cancer only, (ii) censoring at another tumour if applicable or (iii) stratifying by the Rotterdam Study subcohort all showed similar results (Table 1). Notably, also i*n situ* breast cancer showed a statistically significant association with the PRS_{313}, HR per SD=1.43, 95%CI [1.01-2.01], p=0.042.

Association analyses for breast cancer and percentiles of the PRS_{313} showed that the HR-estimates were in line with the HR predicted when a continuous PRS_{313} is assumed, under a log-linear model (Figure 1, Table 1).

During follow-up, 744 women developed another tumour than breast cancer without evidence for association with the PRS_{313} (HR per SD=1.05, 95%CI [0.98-1.12], p-value=0.195).

Age-varying effect

Extension of the Cox model allowing for age-dependent regression coefficients showed that the performance of the PRS_{313} decreased with increasing inclusion age, with the HRs per SD declining from 2.74, 95%CI [1.72-4.37] for women included before age 50, to 1.74, 95%CI [1.52-2.00] for women included between 50 and 75 (p_{diff}=0.066). The HR for women included after age 75 was 1.29, 95%CI [1.08-1.55], and the p-value of the difference with respect to the youngest group was 0.003 (Table 1).

Table 1: Results of the association analyses between breast cancer and the PRS_{313}

		n Included	n Events	HR	95% CI	p-value	C-statistic[c]	95% CI
Main analyses		6522	320	1.56	1.40-1.74	$2.47x10^{-15}$		
Age category for discriminative ability of the PRS	**<60**	2175	104				0.632	0.58-0.69
	60-70	2174	128				0.673	0.61-0.73
	≥70	2173	88				0.562	0.48-0.62
Sensitivity analyses	**Invasive BC only**	6522	290[a]	1.57	1.40-1.77	$1.34x10^{-14}$		
	In situ BC only	6522	34	1.43	1.01-2.01	0.042		
	Censored at other tumour	6402[b]	298	1.54	1.37-1.73	$1.88x10^{-13}$		
	Stratified by RS cohort	6522	320	1.56	1.40-1.75	$1.92x10^{-15}$		
Percentage of the PRS	**0-10%**	637	17	0.59	0.34-1.01	0.053		
	10-20%	636	16	0.58	0.33-1.01	0.053		
	20-40%	1283	42	0.73	0.49-1.09	0.120		
	40-60%	1298	57	1.00	ref	ref		
	60-80%	1325	85	1.49	1.07-2.09	0.019		
	80-90%	656	36	1.28	0.84-1.94	0.251		
	90-100%	687	67	2.37	1.66-3.37	$1.73x10^{-06}$		
Age category for time-varying analyses	**<50**	224	2	2.74	1.72-4.37	$2.23x10^{-05}$		
	50-75	5104	197	1.74	1.52-2.00	$2.21x10^{-15}$		
	>75	4032	121	1.29	1.08-1.55	0.005		

Abbreviations: BC, Breast Cancer; CI, Confidence Interval; HR, Hazard Ratio; PRS, Polygenic Risk Score; RS, Rotterdam Study.

[a] 4 women developed an invasive breast tumour after development of an *in situ* breast tumour.

[b] 120 women were excluded from analyses because they developed another tumour before inclusion in the Rotterdam study.

[c] The corresponding differences in C-statistic were for women with inclusion age 60-70 versus age <60: 0.041, 95%CI [-0.05-0.12]; for women with inclusion age 60-70 versus age ≥70: 0.111, 95%CI [0.02-0.21]; for the women with inclusion age <60 versus age ≥70: 0.070, 95%CI [-0.01-0.18].

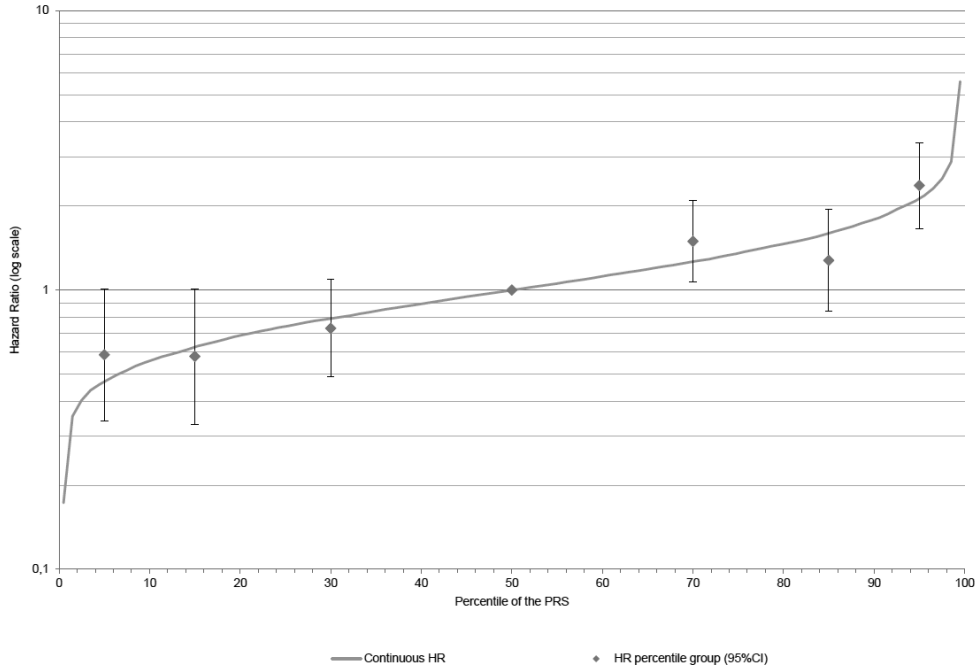

Figure 1: Association with the PRS_{313} and breast cancer risk
Plot of the HR for the association between the PRS_{313} and breast cancer risk based on PRS_{313} percentiles. The PRS_{313} percentile groups are 0-10%, 10-20%, 20-40%, 40-60% (reference), 60-80%, 80-90%, 90-100% based on the distribution in unaffected women. The numbers and corresponding effect sizes are shown in Table 1. The solid line represents the continuous distribution based on the per SD effect size of the PRS_{313}.
Abbreviations: CI, Confidence Interval; HR, Hazard Ratio; PRS, Polygenic Risk Score.

Clinical validity of BOADICEA V5

For these analyses, we selected 4,377 women with an age of inclusion under 70 years. Of these, 163 developed breast cancer within 10 years after inclusion, of whom 142 invasive. The median follow-up in this subcohort was 10 years (range 0.03 – 10 years), and the cumulative incidence of breast cancer was 4.4% (95%CI [3.7%-5.1%]). The distributions of 10-year cumulative risk scores under different models are shown in Figure S4. Irrespective of the variables included, BOADICEA underestimated the observed risk of 4.4% (Table 2). Accordingly, while using age and PRS_{313} seems to result in the best calibration (Figure S4C), it underestimated the observed risks in the higher risk categories. The highest discriminative ability was found for the model with age, PRS_{313} and all available risk factors (0.653, 95%CI [0.60-0.70]), henceforth the "full" model. The PRS_{313} was the strongest factor contributing to discrimination, relative to age and other risk factors (Table 2).

Using the full model and a threshold of 2.5% 10-year breast cancer risk, which approximates the risk of women entering the age-based population screening program in The Netherlands, 101 cases (62% of incident cases) occurred in a screening-group of 1,956 women (45% of total) and 2,421 women would not be screened, in which 62 breast cancers occurred (Figure 2; Table 3). Using the PRS_{313} and age only, 130 cases (80% of incident cases) occurred in a screening-group of 2,863 women (65% of total); 1,481 women would not be screened, in which 33 breast cancers occurred. In Figure S6 the percentages of incident breast cancer cases and unaffected women are shown for different category thresholds. For both models, the invasive cancers in the group selected for screening were more likely to be of lower grade compared to the cancers in the non-screened group (Table 3). The reverse effect was found for *in situ* cancers.

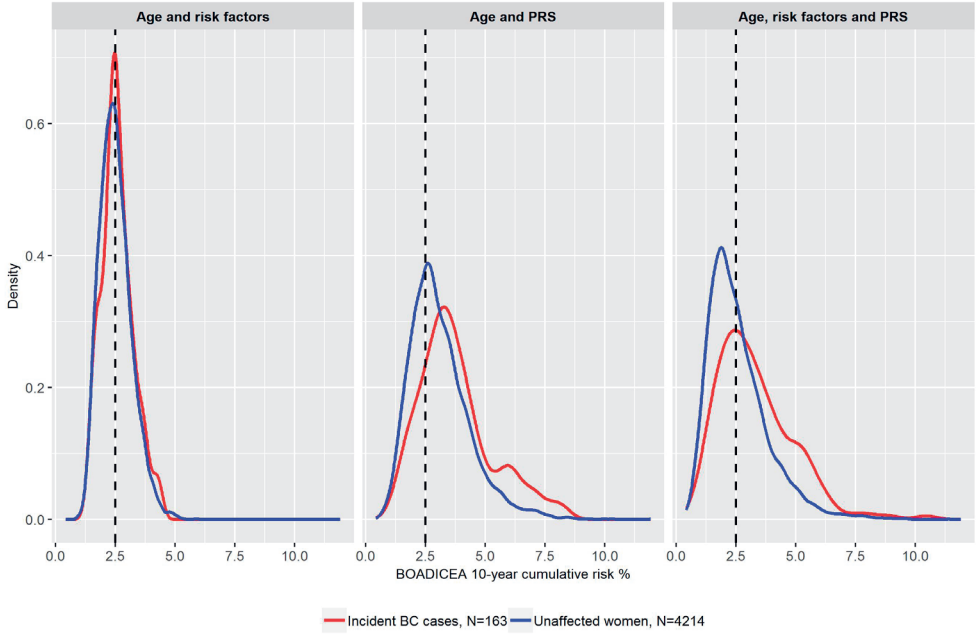

Figure 2: Cumulative 10-year breast cancer risk distribution predicted by BOADICEA
Density plots of the cumulative 10-year risk calculated by BOADICEA for unaffected women and incident breast cancer cases. Including age and risk factors (left), including age and the PRS_{313} (middle) and the full model including age, risk factors and the PRS_{313}. The dashed line shows the threshold of a 10-year risk of 2.5%.
Abbreviations: BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; PRS, Polygenic Risk Score.

Table 2: Range and discriminative ability of the cumulative 10-year breast cancer risk scores calculated with BOADICEA

Variables included	Mean % (range)		C-statistic	95%CI
	Unaffected women	BC cases[a]		
Age	3.0 (2.2-3.6)	2.9 (2.2-3.6)	0.531	0.50-0.58
Age, risk factors	2.5 (1.0-5.9)	2.6 (1.4-4.3)	0.558	0.52-0.60
Age, PRS_{313}	3.1 (0.6-11.9)	3.8 (1.2-8.3)	0.636	0.59-0.68
Age, risk factors, PRS_{313}	2.6 (0.4-11.4)	3.3 (0.9-10.5)	0.653	0.60-0.70

Abbreviations: BC, Breast Cancer; CI, Confidence Interval; PRS, Polygenic Risk Score
[a] Women who developed BC within 10 years of follow up.

Table 3: Numbers and percentages of women per 10-year risk category

				10-year risk category based on BOADICEA			
			Total	Including age and PRS		Including age, risk factors and PRS	
				<2.5%	>2.5%	<2.5%	>2.5%
Unaffected women	All		4214	1481 (35%)	2733 (65%)	2359 (56%)	1855 (44%)
Incident BC cases	All		163	33 (20%)	130 (80%)	62 (38%)	101 (62%)
	invasive BC	All	142	30 (21%)	112 (79%)	52 (37%)	90 (63%)
		Grade 1	19	2 (11%)	17 (89%)	3 (16%)	16 (84%)
		Grade 2	38	7 (18%)	31 (82%)	12 (32%)	26 (68%)
		Grade 3	43	13 (30%)	30 (70%)	21 (49%)	22 (51%)
		Unknown	42	8 (19%)	34 (81%)	16 (38%)	26 (62%)
	In situ BC	All	21	3 (14%)	18 (86%)	10 (48%)	11 (52%)
		Grade 1	3	2 (67%)	1 (33%)	2 (67%)	1 (33%)
		Grade 2	3	1 (33%)	2 (67%)	2 (67%)	1 (33%)
		Grade 3	13	0 (0%)	13 (100%)	5 (38%)	8 (62%)
		Unknown	2	0 (0%)	2 (100%)	1 (50%)	1 (50%)

Abbreviations: BC, Breast Cancer; BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; PRS, Polygenic Risk Score

Discussion

Many risk factors for breast cancer, both genetic and non-genetic, have been identified the past decades[18, 19]. Increasingly, these are being integrated into computational models that allow personalised breast cancer risk assessment, which has potential application beyond current practice of genetic testing in family cancer clinics[8, 9, 20]. The BOADICEA algorithm is among the most comprehensive risk models presently available for breast cancer risk assessment[10]. Here, we validated the most recent version of this model in a large prospective population-based Dutch cohort of women above 45 years, which hasn't been part of the previously published BCAC study[11]. Unsurprisingly, the best discrimination was achieved after inclusion of all available risk factors, with the largest contribution deriving from the PRS_{313}. The PRS_{313} was significantly associated with breast cancer, with a similar effect size as in other prospective series of different geographic origin[11], demonstrating its robustness and potential application to the Dutch population.

The PRS_{313} improved the discriminatory ability from 0.531 to 0.636, compared with a model using age only, which could only be marginally improved further (to 0.653) by adding lifestyle, reproductive factors and anthropometric data. This is in line with previous research, showing that the variance explained by the risk factors are modest compared to the PRS_{313} risk stratification[10, 21]. Results of the calibration showed that BOADICEA underestimated the observed risks, especially in the higher categories of risk. One possible explanation is that BOADICEA v5 uses the population breast cancer incidences of the United Kingdom as baseline risk, which are slightly lower than those in the Netherlands[1]. But more importantly, data on family history, mammographic density and rare high-risk variants in *BRCA1* and *BRCA2* were lacking in our cohort. In another prospective validation study of a previous version of BOADICEA in two cohorts of women from Australia, Canada, and the USA, information on family history and *BRCA1/2* carrier status, but not the PRS_{313}, was available, and here, BOADICEA overestimated 10-year cumulative risks in the highest risk quantile[9]. Possibly, the missing data on family history and *BRCA1/2* status in the Rotterdam Study were in fact more prevalent than modelled by BOADICEA. Our calibration results indicate that for proper use in the general population, information on family history may be important.

We illustrated the potential impact of the model in detecting breast cancer in a population-screening setting in which women would participate based on their individual risk. In this illustration, the PRS_{313} alone would have detected more cases than the full BOADICEA model, but would also have identified a larger screening group. Apparently, women in the Rotterdam Study have on average fewer non-genetic risk factors compared to the total population, which on average slightly modifies their risk in a downward direction. The PROCAS study used the Tyrer-Cuzick model with mammographic density and risk factors,

combined with a PRS based on 18 SNPs[22]; they found 82% of the cases to occur in 68% of women with a 10-year breast cancer risk above 2%, i.e., very similar to what we found with the PRS_{313} alone.

Remarkably, we found the proportion of low grade invasive tumours to be higher in those with a 10-year risk >2.5%, compared to those with lower risks. Screen-detected invasive cancers are more likely of lower grade and stage[23]. Our cohort data did not include information on whether incident breast cancers were screen-detected or not, hence we cannot exclude that high-risk women disproportionally self-selected for mammographic screening, which could explain this bias. In contrast, for the *in situ* carcinomas, more high grade tumours were found in the >2.5% 10-year risk group compared to those with lower risks. Histological grade of Ductal Carcinoma In Situ (DCIS) has been suggested to be one of six factors associated with subsequent development of invasive disease[24], albeit not very strongly so. It remains possible that the PRS_{313} is more strongly associated with low grade invasive breast cancer than with higher grades, as observed for some individual variants[25, 26], and inversely so for DCIS. It will be important to replicate this in larger studies to inform the evaluation of the cost-effectiveness of a risk-based versus age-based entry of the population-screening[7].

Although PRS development studies have included only invasive breast cancer[11, 27], in our cohort the PRS_{313} is associated with i*n situ* breast cancer as well, with a non-significantly lower effect-size than for invasive breast cancer. This corresponds well with a previously reported association of an 18-SNP-based PRS[22] and with previous results showing that the association of 51 of the 76 investigated breast cancer loci with DCIS is in the same direction as for invasive breast cancer[28]. Although BOADICEA is presented as a model that predicts invasive breast cancer[10], these results suggest it might also predict *in situ* breast cancer. Larger studies are needed to confirm this and provide more accurate risk estimates, specifically in the setting of population screening programs.

As in previous studies[11, 27], we found that the effect-size of the PRS for breast cancer declined with increasing age. While this is not yet modelled in BOADICEA, this could be important to consider for women under the age of 50 who are at this moment not eligible for population breast cancer screening in the Netherlands, because our results suggest that using the overall HR would be underestimating risk in this age group.

In the Rotterdam Study, malignancies other than breast cancer are also recorded. We found no evidence for association of the PRS_{313} with these cancers, suggesting it specifically predicts breast cancer. Another prospective study also reported no association between other types of cancer and a sum of breast cancer risk alleles at 72 loci[29]. Because we only

analysed all other tumours combined, we cannot exclude that the PRS_{313} has an association with one specific type of other cancer.

A strength of our study is the prospective population-based study design, including all women in a specified locale near Rotterdam. Because of the high response rate (>70%) it is a good representation of the Dutch population in that age category[13]. Furthermore, for a large group of women, there is extensive follow up of up to 25 years.

Besides that information on mammographic density and family history was lacking, another limitation of our study is the unknown ER-status of the breast tumours, precluding the analysis of ER-positive and ER-negative disease separately. Furthermore, to evaluate the introduction of risk-based entry into population-screening, establishing the detection rate of breast cancers below the age of 50 would have been relevant, which was not possible in our older cohort of women. Finally, we excluded nearly 25% of all women in the Rotterdam Study because no genotyping data were available. Declining blood-donation for DNA extraction did not lead to differences in the basic characteristics between the genotyped and non-genotyped groups. Therefore, if a selection bias was present, we believe this bias would be small.

In summary, the PRS_{313} replicates robustly in the Dutch population and the discriminative power of the BOADICEA model seems appropriate for implementation into breast cancer prevention programs, such as those currently ongoing in cancer family clinics in many countries worldwide. However, application to the general population would require recalibration of BOADICEA to address underestimation in the higher risk categories. Although the Rotterdam Study design precluded analysis of breast cancer-specific mortality, our evaluation of clinical validity provides first insights into how a risk-based entry could impact the efficacy of the breast cancer population screening program in the Netherlands.

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Disclosure of potential conflicts of interest

The authors declare no conflicts of interest

References

1. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *European journal of cancer (Oxford, England : 1990)*. Nov 2018;103:356-387. doi:10.1016/j.ejca.2018.07.005
2. van der Waal D, Verbeek AL, den Heeten GJ, Ripping TM, Tjan-Heijnen VC, Broeders MJ. Breast cancer diagnosis and death in the Netherlands: a changing burden. *European journal of public health*. Apr 2015;25(2):320-4. doi:10.1093/eurpub/cku088
3. Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M. The benefits and harms of breast cancer screening: an independent review. *British journal of cancer*. Jun 11 2013;108(11):2205-40. doi:10.1038/bjc.2013.177
4. Ripping TM, Verbeek AL, Fracheboud J, de Koning HJ, van Ravesteyn NT, Broeders MJ. Overdiagnosis by mammographic screening for breast cancer studied in birth cohorts in The Netherlands. *International journal of cancer*. Aug 15 2015;137(4):921-9. doi:10.1002/ijc.29452
5. The benefits and harms of breast cancer screening: an independent review. *Lancet (London, England)*. Nov 17 2012;380(9855):1778-86. doi:10.1016/s0140-6736(12)61611-0
6. Hubbard RA, Kerlikowske K, Flowers CI, Yankaskas BC, Zhu W, Miglioretti DL. Cumulative probability of false-positive recall or biopsy recommendation after 10 years of screening mammography: a cohort study. *Annals of internal medicine*. Oct 18 2011;155(8):481-92. doi:10.7326/0003-4819-155-8-201110180-00004
7. Pashayan N, Morris S, Gilbert FJ, Pharoah PDP. Cost-effectiveness and Benefit-to-Harm Ratio of Risk-Stratified Screening for Breast Cancer: A Life-Table Model. *JAMA oncology*. Nov 1 2018;4(11):1504-1510. doi:10.1001/jamaoncol.2018.1901
8. Cintolo-Gonzalez JA, Braun D, Blackford AL, et al. Breast cancer risk models: a comprehensive overview of existing models, validation, and clinical applications. *Breast cancer research and treatment*. Jul 2017;164(2):263-284. doi:10.1007/s10549-017-4247-z
9. Terry MB, Liao Y, Whittemore AS, et al. 10-year performance of four models of breast cancer risk: a validation study. *The Lancet Oncology*. Apr 2019;20(4):504-517. doi:10.1016/s1470-2045(18)30902-1
10. Lee A, Mavaddat N, Wilcox AN, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. *Genetics in medicine : official journal of the American College of Medical Genetics*. Jan 15 2019;21(8):1708-1718. doi:10.1038/s41436-018-0406-9
11. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *American journal of human genetics*. Jan 3 2019;104(1):21-34. doi:10.1016/j.ajhg.2018.11.002
12. IKNL. Netherlands Comprehensive Cancer Organisation: Oncoline Mammacarcinoom. 2019. Available from www.oncoline.nl/richtlijn/item/index.php?pagina=/richtlijn/item/pagina.php&richtlijn_id=885. Accessed December, 2019;
13. Ikram MA, Brusselle G, Ghanbari M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. *European journal of epidemiology*. May 4 2020;doi:10.1007/s10654-020-00640-5

14. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nature genetics*. Oct 2016;48(10):1279-83. doi:10.1038/ng.3643
15. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature*. Oct 1 2015;526(7571):68-74. doi:10.1038/nature15393
16. Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Statistics in medicine*. May 10 2011;30(10):1105-17. doi:10.1002/sim.4154
17. R_Core_Team_(2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
18. Rojas K, Stuckey A. Breast Cancer Epidemiology and Risk Factors. *Clinical obstetrics and gynecology*. Dec 2016;59(4):651-672. doi:10.1097/grf.0000000000000239
19. Lakeman IMM, Schmidt MK, van Asperen CJ, Devilee P. Breast Cancer Susceptibility—Towards Individualised Risk Prediction. journal article. *Current Genetic Medicine Reports*. June 01 2019;7(2):124-135. doi:10.1007/s40142-019-00168-5
20. Turnbull C, Sud A, Houlston RS. Cancer genetics, precision prevention and a call to action. *Nature genetics*. Sep 2018;50(9):1212-1218. doi:10.1038/s41588-018-0202-0
21. Maas P, Barrdahl M, Joshi AD, et al. Breast Cancer Risk From Modifiable and Nonmodifiable Risk Factors Among White Women in the United States. *JAMA oncology*. Oct 1 2016;2(10):1295-1302. doi:10.1001/jamaoncol.2016.1025
22. van Veen EM, Brentnall AR, Byers H, et al. Use of Single-Nucleotide Polymorphisms and Mammographic Density Plus Classic Risk Factors for Breast Cancer Risk Prediction. *JAMA oncology*. Apr 1 2018;4(4):476-482. doi:10.1001/jamaoncol.2017.4881
23. Autier P, Boniol M, Koechlin A, Pizot C, Boniol M. Effectiveness of and overdiagnosis from mammography screening in the Netherlands: population based study. *BMJ (Clinical research ed)*. Dec 5 2017;359:j5224. doi:10.1136/bmj.j5224
24. Visser LL, Groen EJ, van Leeuwen FE, Lips EH, Schmidt MK, Wesseling J. Predictors of an Invasive Breast Cancer Recurrence after DCIS: A Systematic Review and Meta-analyses. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. May 2019;28(5):835-845. doi:10.1158/1055-9965.Epi-18-0976
25. Garcia-Closas M, Hall P, Nevanlinna H, et al. Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. *PLoS genetics*. 4/25/2008 2008;4(4):e1000054. In File.
26. Purrington KS, Slettedahl S, Bolla MK, et al. Genetic variation in mitotic regulatory pathway genes is associated with breast tumor grade. *Hum Mol Genet*. 11/15/2014 2014;23(22):6034-6046. In File.
27. Mavaddat N, Pharoah PD, Michailidou K, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *JNatlCancer Inst*. 5/2015 2015;107(5)Not in File. doi:djv036 [pii];10.1093/jnci/djv036 [doi]

28. Petridis C, Brook MN, Shah V, et al. Genetic predisposition to ductal carcinoma in situ of the breast. *Breast cancer research : BCR*. Feb 17 2016;18(1):22. doi:10.1186/s13058-016-0675-7
29. Naslund-Koch C, Nordestgaard BG, Bojesen SE. Common breast cancer risk alleles and risk assessment: A study on 35,441 individuals from the Danish general population. *Annals of oncology : official journal of the European Society for Medical Oncology*. Oct 13 2016;doi:10.1093/annonc/mdw536

Supplementary figures and tables

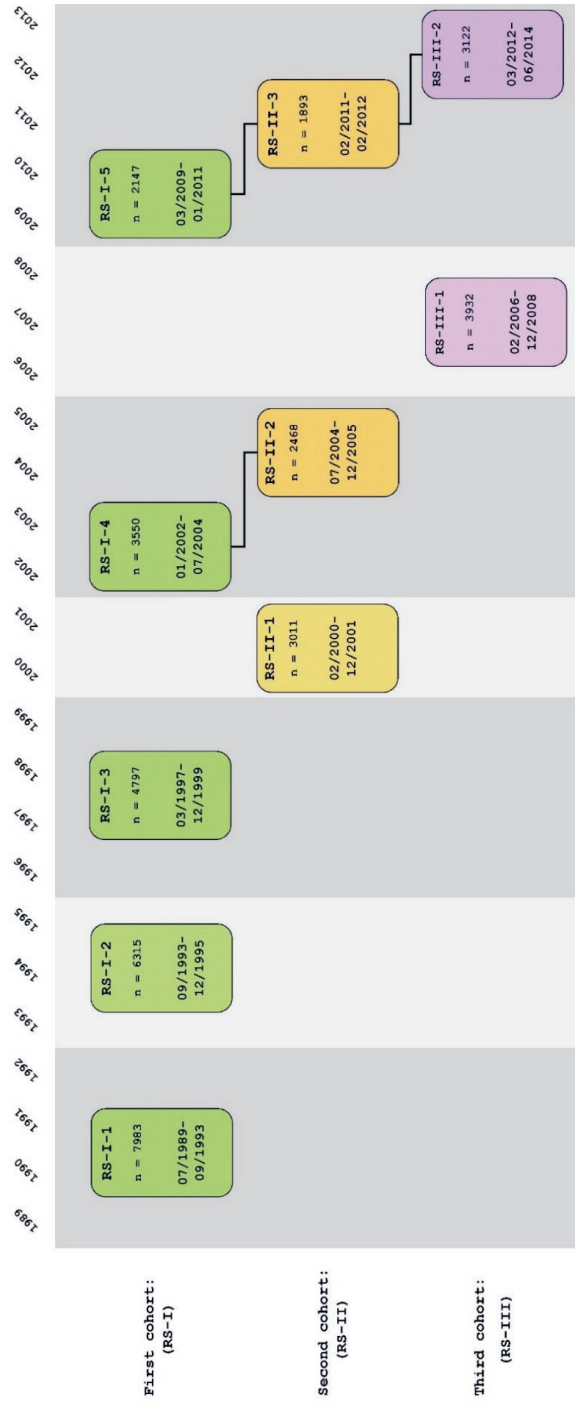

Figure S1: Time points of the first and follow up questionnaires for the Rotterdam Study cohorts
Diagram of the included individuals and contact moments for the Rotterdam Study. Green boxes: RS-I cohort, yellow boxes: RS-II cohort; purple boxes: RS-III cohort. A more elaborate figure with information about the follow up after 2013 is published by Ikram et al.[1].

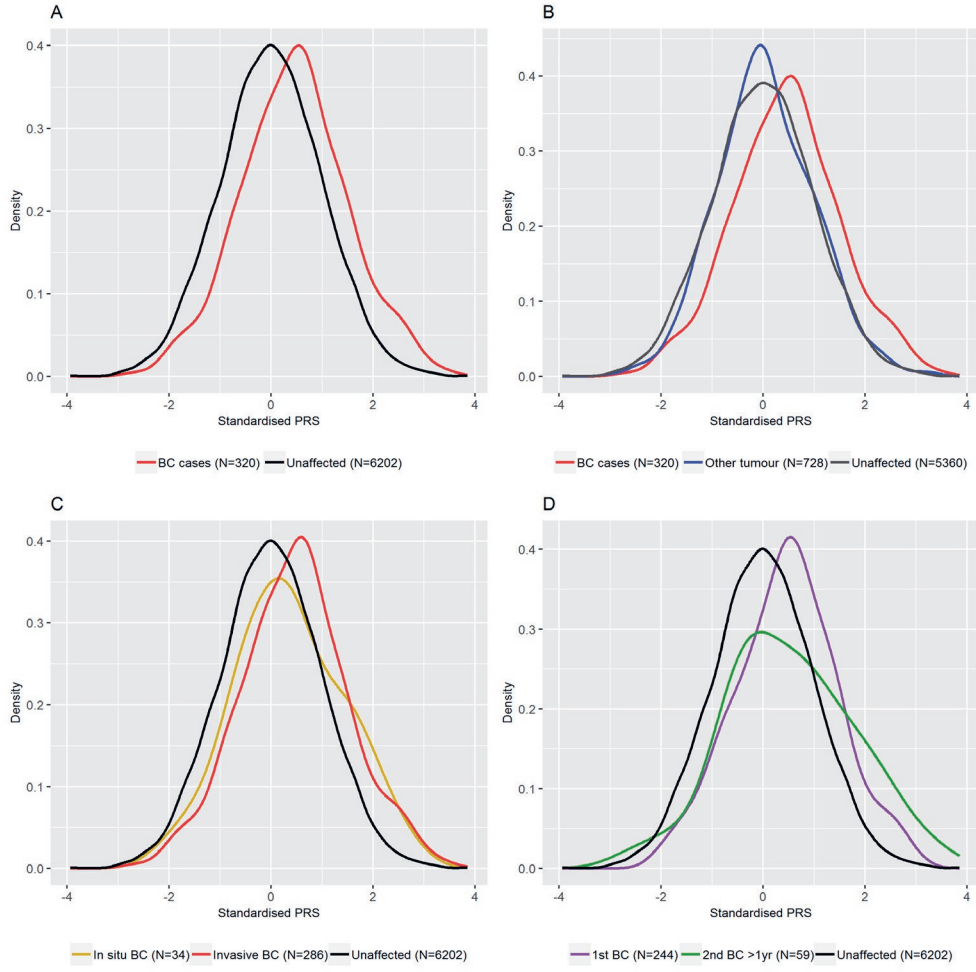

Figure S2: Distribution curves of the PRS_{313} in the Rotterdam Study cohort

Abbreviations: BC, Breast Cancer; PRS, Polygenic Risk Score

The standardised PRS_{313} was plotted against the density for different groups in the Rotterdam Study. (A) incident BC cases and unaffected women; (B) incident BC cases, unaffected women who developed another type of tumour and unaffected women who did not develop another type of tumour. Women who developed another type of tumour before inclusion in the Rotterdam Study were excluded (N=114); (C) invasive incident BC cases, *in situ* incident BC cases and unaffected women; (D) Incident BC cases who developed one breast tumour, incident BC cases who developed a second primary breast tumour after one year and unaffected women. Women who developed a second primary breast tumour within one year were excluded (N=17). Unaffected women include all those that did not develop BC.

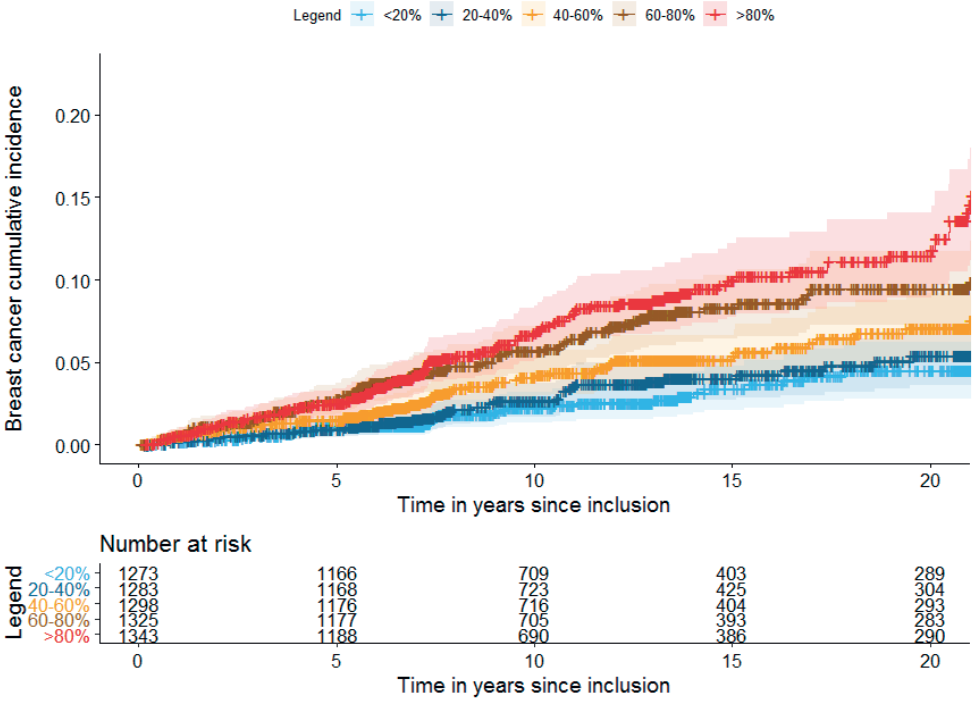

Figure S3: Cumulative breast cancer incidence in the Rotterdam study stratified on PRS_{313} quintiles

Abbreviations: PRS, Polygenic Risk Score.

Kaplan Meier plot for the cumulative breast cancer incidence since the time of inclusion in the Rotterdam Study. The cohort is stratified in quintiles of the PRS_{313}, based on the distribution of unaffected women in the cohort.

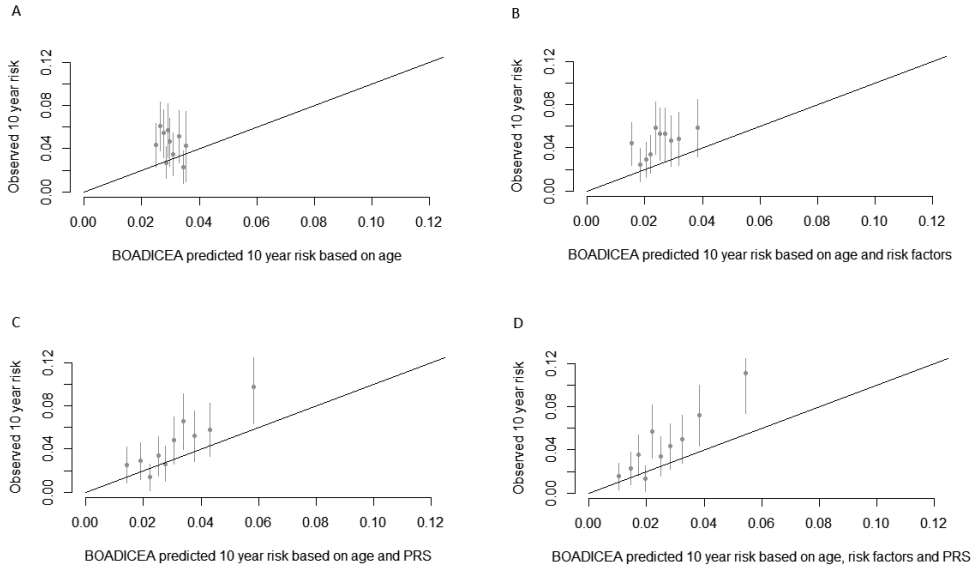

Figure S4: Calibration plots of the predicted 10-year risk based on BOADICEA and the observed risk in the Rotterdam Study cohort

Abbreviations: BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; PRS, Polygenic Risk Score.

10-year cumulative BC risks were calculated for all women included in the Rotterdam Study before the age of 70 years, using BOADICEA v5. The difference between the observed and predicted risk is shown per decile of the predicted risk, including 95% confidence intervals, for different sets of included variables. Using age only (A), age and risk factors (B), age and the PRS (C) and age, risk factors, and the PRS (D).

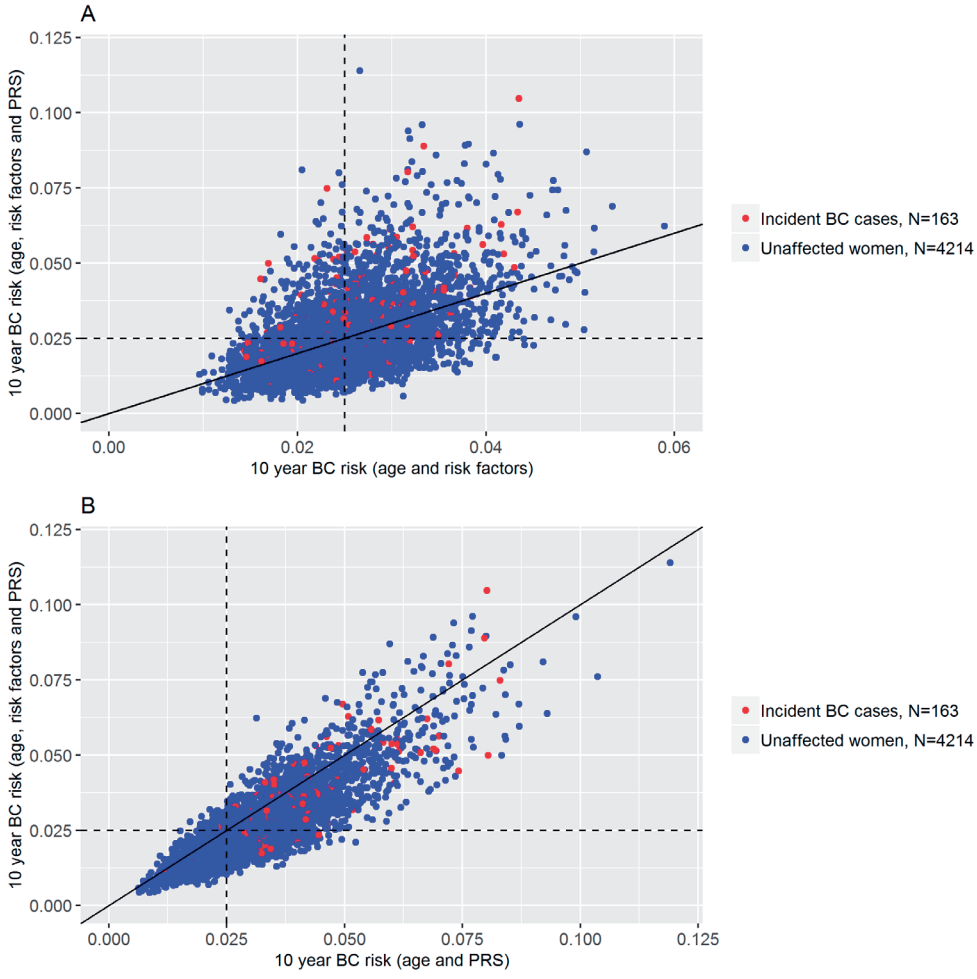

Figure S5: Change in 10-year risk by adding risk factors or the PRS_{313} in the BOADICEA model
Abbreviations: BC, Breast Cancer; BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; PRS, Polygenic Risk Score.
10-year cumulative BC risks were calculated for all women included in the Rotterdam Study before the age of 70 years, using BOADICEA v5. Women were considered as incident BC cases if they developed BC within 10 years of follow up (shown in red). (A) Risk-change by adding the PRS_{313} in the BOADICEA model (y-axis) including age and risk factors (x-axis). (B) Risk-change by adding risk factors in the BOADICEA model (y-axis) including age and the PRS_{313} (x-axis).

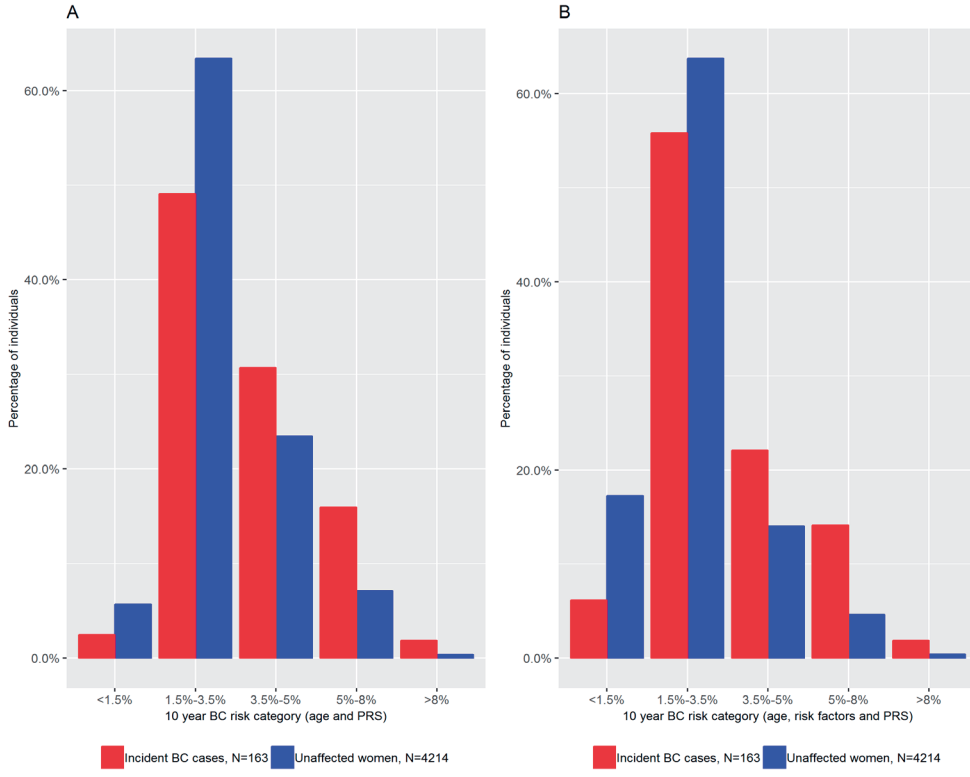

Figure S6: Percentage of unaffected women and incident breast cancer cases in different 10-year risk categories

Abbreviations: BC, Breast Cancer; BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; PRS, Polygenic Risk Score.

Bar plot of the percentages of women assigned to the different 10-year cumulative BC risk categories (<1.5%; 1.5%-3.5%; 3.5%-5%; 5%-8%; >8%) as calculated with BOADICEA v5 using two sets of variables. Including age and the PRS_{313} (A) and including age, risk factors and the PRS_{313} (B). These risks were calculated for all women included in the Rotterdam Study below the age of 70 years. Women were considered affected if they developed BC within 10 years of follow up.

Table S1: Characteristics of the Rotterdam Study cohort

		Total cohort		Subcohort[a]	
		Unaffected	Incident BC	Unaffected	Incident BC
Number		6202	320	4214	163
Rotterdam Study cohort	**RS-I**	3536	227	1821	152
	RS-II	1057	59	796	50
	RS-III	1609	34	1525	33
Birth cohort	**<1900**	54	1	0	0
	1900-1910	487	9	0	0
	1910-1920	996	46	0	0
	1920-1930	1441	106	976	77
	1930-1940	1293	97	1235	97
	1940-1950	1087	42	1087	82
	1950-1960	811	18	811	18
	1960	33	1	33	1
Age at inclusion	**Mean**	66.1	65	59.9	60.4
	Range	45.8-99.2	45.8-96.3	45.8-70.0	45.8-70.0
Age at diagnosis	**Mean**	-	72.7	-	65.3
	Range	-	48-100	-	48.0-79.0
Invasiveness first BC	**Invasive**	-	286	-	142
	In situ	-	34	-	21
Asynchronous second BC[b]	**All**	-	59	-	44
	Invasive	-	59	-	44
	In situ	-	0	-	0
Other incident tumour[c]		728	16	450	13
Risk factors					
Height in cm	**Mean**	162.3	163.0	164.0	164.3
	Unknown	137 (2%)	5 (2%)	9 (0.2%)	3 (2%)
Alcohol use in grams per day	**Mean**	6.3	7.1	6.8	6.8
	Unknown	506 (8%)	11 (3%)	742 (18%)	34 (21%)
Age menarche	**Mean**	13.5	13.3	13.4	13.3
	Unknown	317 (5%)	11 (3%)	102 (2%)	4 (2%)
Age menopause	**Mean**	48.8	49.2	48.6	49.4
	Unknown	473 (8%)	24 (8%)	255 (6%)	15 (9%)
	Premenopausal	-	-	187	5
Number of children	**0**	482	25	408	15
	1	811	39	642	22
	2	1819	93	1549	52
	>2	1443	80	1031	41
	Unknown	1647 (27%)	83 (26%)	584 (14%)	33 (20%)
Age at first childbirth	**Mean**	25.2	25.2	25.0	25.6
	Unknown[d]	47 (1%)	5 (2%)	603 (14%)	34 (21%)

Use of oral contraception	**Never**	2346	137	1238	50
	Ever	2774	126	2665	90
	Unknown	1082 (17%)	57 (18%)	311 (7%)	23 (14%)
Use of hormone replacement therapy	**Never**	5050	254	3416	128
	Ever	994	62	758	32
	Unknown	158 (2.5%)	4 (1%)	40 (1%)	3 (2%)
Body Mass Index	**Mean**	27.0	27.7	27.0	27.8
	Unknown	141 (2%)	5 (2%)	38 (1%)	3 (2%)
Standardised PRS_{313}	**Mean**	0	0.45	-0.01	0.57
	SD	1.00	1.05	1.00	1.02

Abbreviations: BC, Breast Cancer; PRS, Polygenic Risk Score; RS, Rotterdam Study; SD, Standard Deviation.

[a] Subcohort of women with an age of inclusion in the Rotterdam Study up to age 70

[b] Development of a second primary breast tumour at least one year after the first primary breast tumour.

[c] For women who developed BC during follow up, other tumours were only reported in this study if the other tumour was diagnosed before the BC diagnosis.

[d] For women known to have children.

Table S2: 313 breast cancer associated variants included in the Polygenic Risk Score
See online material. First 7 columns of the table are published by Mavaddat et al.[2]

Table S3: Number of included women diagnosed with other type of tumours

ICD10	Tumour description[a]	Unaffected women	Incident BC cases[b]	Total
C00	Lip	5		5
C02	Tongue	2		2
C03	Gum	1		1
C04	Floor of mouth	1		1
C05	Palate	2		2
C06	Mouth	2		2
C08	Major salivary glands	1		1
C09	Tonsil	2		2
C10	Oropharynx	1		1
C15	Oesophagus	27		27
C16	Stomach	21		21
C17	Small intestine	3	1	4
C18	Colon	90	1	91
C19	Rectosigmoid	33	2	35
C20	Rectum	38	1	39
C21	Anus and anal canal	5		5
C22	Liver and intrahepatic bile ducts	8		8
C23	Gallbladder	2		2
C24	Biliary tract	6		6
C25	Pancreas	44		44
C26	Digestive organs	4		4
C32	Larynx	1		1
C34	Bronchus & lung	112		112
C39	Respiratory system and intrathoracic organs	1		1
C40	Bone and articular cartilage of limbs	2		2
C43	Melanoma	27	2	29
C45	Mesothelioma	4		4
C48	Retroperitoneum and peritoneum	1		1
C49	Connective and soft tissue	3		3
C51	Vulva	6		6
C52	Vagina	1		1
C53	Cervix uteri	10	1	11
C54	Corpus uteri	48	2	50
C56	Ovary	24		24
C57	Female genital organs	1		1
C64	Kidney, except renal pelvis	16	1	17
C65	Renal pelvis	4		4
C66	Ureter	1		1
C67	Bladder	24	1	25
C69	Eye and adnexa	5		5
C70	Meninges	1		1
C71	Brain	13		13
C73	Thyroid gland	4	1	5
C80	Malignant neoplasm unspecified	37	1	38

C81	Hodgkin lymphoma	1		1
C82	Follicular lymphoma	6	1	7
C83	Non-follicular lymphoma	13		13
C84	Mature T/NK-cell lymphomas	2		2
C85	Non-Hodgkin lymphoma	11		11
C88	Immunoproliferative diseases	1		1
C90	Multiple myeloma and malignant plasma cell neoplasms	19	1	20
C91	Lymphoid leukaemia	12		12
C92	Myeloid leukaemia	17		17
C93	Monocytic leukaemia	2		2
Total		728	16	744

Abbreviations: BC, Breast Cancer; ICD, International Classification of Diseases and Related Health Problems

[a] ICD10 tumour description[3]

[b] Other tumours are only reported if a woman developed this tumour before the BC diagnosis

Table S4: Descriptives for the standardised PRS_{313}

		Number	Mean	SD	SE	95% CI
Unaffected	**Total**	6202	0.00	1.00	0.01	-0.02-0.02
	Without other tumour	5360	-0.01	1.01	0.01	-0.03-0.02
	Incident other tumour[a]	728	0.03	0.98	0.04	-0.04-0.10
Incident BC cases	**Total**	320	0.45	1.05	0.06	0.34-0.57
	Invasive BC	286	0.46	1.05	0.06	0.34-0.58
	***In situ* BC**	34	0.36	1.06	0.18	0.00-0.72
	One primary breast tumour	244	0.46	1.00	0.06	0.33-0.59
	Asynchronous second BC[b]	59	0.51	1.27	0.17	0.19-0.84

Abbreviations: BC, Breast Cancer; CI, Confidence Interval; PRS, Polygenic Risk Score; SD, Standard Deviation; SE, Standard Error.

[a] Women who developed another type of tumour before inclusion in the Rotterdam Study were excluded (N=114)

[b] Development of a second primary breast tumour at least one year after the first primary breast tumour.

Supplementary references

1. Ikram MA, Brusselle G, Ghanbari M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. *European journal of epidemiology*. May 4 2020;doi:10.1007/s10654-020-00640-5
2. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *American journal of human genetics*. Jan 3 2019;104(1):21-34. doi:10.1016/j.ajhg.2018.11.002
3. ICD-10 Version:2016. 2016. Available from: https://icd.who.int/browse10/2016/en. Accessed March, 2019;