The path to individualised breast cancer screening

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CHAPTER 1

General introduction

General introduction[2]

Breast cancer burden

Breast cancer is worldwide the most common cancer among women, especially in Western Europe[3], and is responsible for almost 25% of the total cancer burden for women[4]. In 2019 and 2020, respectively 17,148 and 14,935 women were diagnosed with breast cancer in the Netherlands[5]. Most breast cancers are detected by mammographic screening. The remainder by palpation of a breast mass, axillary mass or skin abnormalities[6]. Dependent on the abnormality, an additional ultrasound or biopsy is recommended to differentiate between a benign abnormality (e.g. fibroadenoma, ductal hyperplasia), in situ cancer or invasive breast cancer[7, 8]. In situ cancers are classified as low, medium or high grade by histological features[9]. Classification of invasive breast cancer, which can guide treatment options and estimate prognosis, is based on histological type (pathologic growth pattern), grade and tumour stage. More than 20 histological types of breast cancer are known of which the most common are infiltrating duct carcinomas, no special type (70-80%) and invasive lobular carcinomas (~10%)[10]. Tumour grade is a good prognostic factor and includes microscopic assessment of histologic differentiation (tubule formation, nuclear pleomorphism, and proliferation). Tumour stage combines data on tumour size, nodal status and distant metastasis. The most common sites of distant metastasis include the lung, bone and liver. Important for considering hormone therapy is determination of hormone receptor status of the tumour. The majority of breast tumours, about ~75%, express Estrogen Receptor (ER) and/or Progesterone Receptor (PR). Usually, these hormone receptor-positive tumours are low grade and less aggressive. A minority of roughly 15% of breast tumours have overexpression of human epidermal growth factor 2 receptors (HER2), which predict a favourable response to anti-HER2 therapy. However, these tumours are known to be aggressive and have a poor prognosis. Triple-negative breast cancers (i.e., negative for ER, PR and HER2 amplification) comprise about 10% of all breast tumours, are mostly high grade and have a poor prognosis[10].

Breast cancer screening

The high prevalence of breast cancer in the Netherlands equates to an average lifetime risk of 12-13%[7] and provided a strong rationale for a population-screening program that started in 1990. This program invites women every two years for mammography, starting at age 50 and ending at age 75. At age 50 the average 10-year risk to develop invasive breast cancer is approximately 3%, exceeding the threshold at which screening becomes cost-effective[11]. About 63% of all breast cancers in 2019 were detected in women between 50 and 75 years of age[5]. The program has a compliance rate of around 80% and has been demonstrated to cause a decline in mortality rate of approximately 1.7% each year[12]. However, this mortality benefit has been offset by an increasing breast cancer incidence of about twofold[5]. Whether the reduction of mortality can be fully ascribed

to mammographic screening or to improvements of therapeutic options is still under debate. It could be the combination of early diagnosis and therapy[13]. Mammographic screening led to a decrease in the rate of large tumours, and an increase in the detection of small tumours which may represent overdiagnosis[14]. Overdiagnosis is the detection of tumours that, if left untreated, would not have become clinically relevant, mostly Ductal Carcinoma In Situ (DCIS), a non-invasive form of breast cancer. Currently, 13% of the total breast cancer burden in the Netherlands is due to DCIS, while this was about 3% before the start of population screening[5, 9, 15]. Although the majority of DCIS lesions remain indolent, all DCIS are treated with surgery (mostly breast-conserving)[9, 15]. Besides that surgery is resulting in overtreatment of at least some of these lesions, women are labelled as cancer patients and experience substantial psychological distress, which shows the disadvantages of screening. Furthermore, mammographic screening results in a high number of false-positive results[16, 17]. Women attending biannual mammographic screening at age 50, have a cumulative 10-year risk of about 6% for a false-positive result leading to a biopsy[18].

To summarise, secondary prevention by early detection through mammographic screening can reduce mortality, but at the cost of overdiagnosis and the burden of false-positive results[16-18]. Primary prevention by risk reducing mastectomy is in the Netherlands restricted to women at high risk, mainly for *BRCA1/2* pathogenic variant carriers. Stratification of women according to the risk of developing breast cancer could provide a persuasive rationale for surgical intervention as well as improve efficacy of risk–reduction and screening strategies by tailoring starting age and frequency[19, 20].

Breast cancer risk

BOX 1: definition of breast cancer risk
Clinically, definitions such as low, moderate and high breast cancer risk are often used. However, this can reflect relative or absolute risks. For a given relative risk (RR), absolute risk can vary between countries depending on cancer incidences. Another term often used is lifetime risk, which is the absolute risk of breast cancer over the period of a woman's life. Here, we define moderate risk as RR = 2 to 4, high risk as RR > 4, and low or population risk as RR < 2.

To accurately assess a woman's risk, it is important to take all risk factors into account. Having a positive family history is one of the main risk factors for breast cancer. For women with at least one first-degree relative with breast cancer, the risk for developing breast cancer is on average about two-fold compared with women without such a family history[21]. Approximately 25% of this so-called familial relative risk (FRR) is currently explained by (likely) pathogenic variants in a small number of genes, and a further 18% by the currently

known common low risk variants, mostly single nucleotide polymorphisms (SNPs)[22-25]. It is estimated that another 18% is explained by common low risk variants present on SNP arrays used for genotyping in genome-wide association studies, but these have not yet been individually discovered[26] (Figure 1). Besides the familial relative risk, other risk factors such as mammographic density and lifestyle factors are important as well[27, 28].

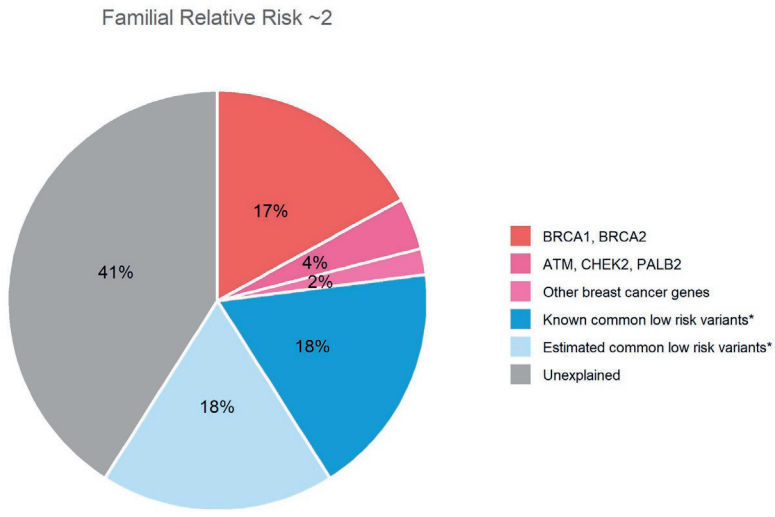

Figure 1. Explained familial relative risk
*For women of European ancestry[26]

Rare genetic variation associated with breast cancer

The definition of "rare" variation is somewhat arbitrary, but is generally taken as to occur in <0.5% of the general population. Indeed, we currently know that some allelic variants in breast cancer susceptibility genes are extremely rare (<0.001%), others moderately rare (~0.1%), or even almost "common" (~1%). In addition, the risks conferred by these variants may vary from less than 2-fold to over 10-fold (Figure 2). Classic linkage analysis in multiple-case families discovered some of these genes, but many were discovered by DNA sequencing of candidate genes. The best-known examples of linkage-detected genes are *BRCA1* and *BRCA2*[29, 30]. Pathogenic variants in either gene, each with a joint allele frequency of ~0.1%, will lead to a high risk of breast and ovarian cancer in women[31, 32]. Other genes, particularly *TP53, PTEN, STK11, CDH1* and *NF1,* were discovered because of their association with typical familial cancer syndromes of which breast cancer is one feature[33-37]. Accordingly, their prevalence in the population is extremely rare. These findings also underscore the pleiotropic effects that some DNA variations display by predisposing to cancers of diverse tissue origin. Yet for most breast cancer genes discovered so far, the most conspicuous "other" cancer with which an association has

been firmly established is ovarian cancer. Another "syndromic" gene is *ATM*; pathogenic variants in *ATM* act in a recessive way to cause ataxia telangiectasia, a neurodegenerative disorder, but heterozygous carriers are at moderately increased risk for breast cancer[38]. The discovery that *BRCA1*, *BRCA2*, and *ATM* are involved in DNA damage repair, and that *BRCA2* is a Fanconi anaemia gene (FANCD1)[39], suggested that other DNA repair genes might also confer breast cancer susceptibility. Sequence analysis of these candidates then led to the discovery of *CHEK2, BARD1, PALB2, NBN,* and *RAD51D*[40-44] as breast cancer genes, although evidence is sometimes limited to specific variants in populations of specific ethnic background[43]. Breast cancer risks in these five genes are generally moderate, with the exception of loss-of-function variants in *PALB2,* which can lead to breast cancer risks comparable to *BRCA2*[43, 45].

There is a long list of genes, including *BRIP1, FANCC, FANCM, MEN1, MRE11A, PPM1D, RAD50, RAD51B, RECQL,* and *XRCC2,* for which an association with breast cancer has been reported in a few studies. Until recently, however, replication in sufficiently large samples of cases and controls and establishment of effect-sizes was still lacking. In 2021, two large population-based case-control studies were published[46, 47] which defined the association of genes often present on commercial breast cancer gene panels with breast cancer risk and provided the most precise risk estimates to date. As expected, robust associations were found for truncating variants in the five well known breast cancer genes, *BRCA1, BRCA2, PALB2, CHEK2*, and *ATM*[46, 47]. Furthermore, truncating variants in *BARD1, RAD51C,* and *RAD51D* were also significantly associated with breast cancer risk in both studies[46, 47], although Hu et al.[47] only detected an association with a ER-negative and triple-negative breast cancer for these genes . An association with truncating variants in respectively *TP53* with overall breast cancer[46], and *CDH1* with ER-positive breast cancer[47] was only found in one of the studies[46, 47]. Modest evidence was demonstrated for an association with truncating variants in *NF1*, *PTEN* and *MSH6*, particularly in ER-negative subtypes. Despite the large sample size, for some genes there is still no consensus about the association with breast cancer risk[46]. A long-standing issue is whether the Lynch syndrome genes (*MLH1, MSH2, MSH6, PMS2*) and *MUTYH* are associated with breast cancer risk. Interpretation of breast cancer incidence in studies of Lynch syndrome families is complicated due to various biases (e.g., ascertainment). Of the lynch syndrome genes, *MSH6* seems to have the highest probability of being associated with breast cancer risk[46, 48]. More detailed discussions on the association of gene variants and breast cancer and the corresponding risks can be found in reviews by Wendt et al., Easton et al., and Graffeo et al.[43, 44, 49].

Allele frequency and corresponding odds ratio for truncating pathogenic variants in associated breast cancer genes, adapted from Dorling et al.[46]. Genes shown in red are robustly associated with breast cancer (p-value <0.0001). Genes shown in orange were marginally associated with breast cancer risk (p-value <0.05). The frequency and

corresponding odds ratio for breast cancer associated common low risk variants, included in the PRS_{313}, are shown in blue and are adapted from the study performed by Mavaddat et al.[26]

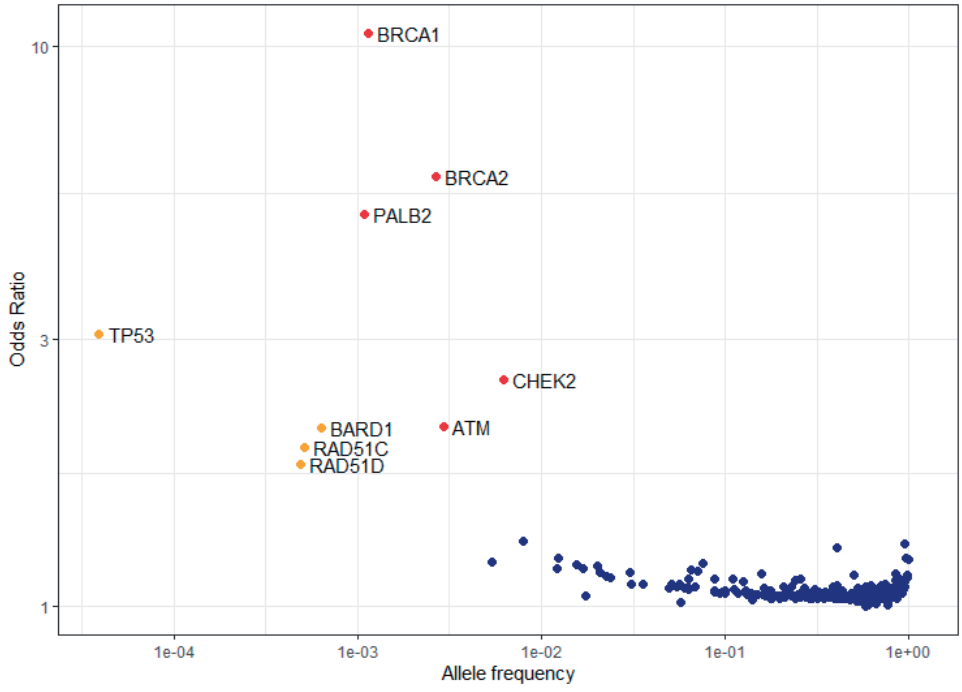

Figure 2. Genetic landscape of breast cancer

Challenges in risk assessment and clinical translation

Once a gene has been robustly associated with breast cancer, other challenges arise that may hamper introduction into the clinic. One is allelic diversity and the notion that different types of variants (e.g., nonsense versus missense changes) might confer different breast cancer risks[43]. For *BRCA1* and *BRCA2*, the effect of mutation-position on the relative risks for breast and ovarian cancer has been firmly established[50]. Furthermore, several missense changes have been identified in *BRCA1* and *BRCA2* that cause much more moderate risks than the typical loss-of-function variants[51, 52]. Conversely, while most pathogenic variants in *ATM* will give an intermediate breast cancer risk, one specific missense mutation (c.7271C>G) seems to reach a higher level of risk. In some studies this risk is even approaching that of *BRCA1/2* pathogenic variants[53, 54]. The presence of allelic diversity in breast cancer genes also highlights the difficulties we are still having with establishing pathogenicity for each variant. This seems straightforward for protein-truncating variants (although exceptions exist[55]), but for many missense and "spliceogenic" variants the impact

on protein function (and, by inference, on cancer risk) is hard to predict. The many *in silico* tools available for this purpose may help classifying these variants, are inexpensive and easy to use, but they still perform modest with respect to clinical standards and, therefore, the predictive power of these tools need to be improved[56]. For some genes, such as *BRCA1* and *BRCA2*, functional assays are developed which show efficacy in variant classification but these are, among other things, time-consuming with a consequence of poor feasibility in daily clinical practice[57]. As a result, many variants detected by sequencing in these genes are still classified as Variants of Uncertain Significance (VUS).

BOX 2: Classification of gene variants

The ACMG has recommended a five-tier classification system, which has been adopted by many countries1. These classes are 1. Benign; 2. Likely Benign; 3. VUS; 4. Likely Pathogenic; 5. Pathogenic. For VUS, the pathogenicity and hence the association with disease risk are unknown, usually because they result in a similarly-shaped amino acid or reside in a part of the gene not essential for its function.

Another challenge is to establish the penetrance of pathogenic variants and the corresponding breast cancer risks with sufficient accuracy. With some exceptions, there is still much uncertainty surrounding the magnitude and precision of the risks conferred by pathogenic variants in the genes. Even in the recently performed large gene-panel studies, the confidence intervals of the associated risks remain wide[46, 47]. One problem underlying this issue is ascertainment bias in the sample used in the analyses. Patient series consisting mostly of women with a positive family history are almost certainly overestimating risk due to enrichment of other risk factors. This is especially true for tumour syndrome genes, investigation of which is usually triggered by the syndrome criteria. For example, the penetrance of *TP53* variants was initially estimated to be very high[58]. But with the introduction of gene panel sequencing, pathogenic variants in *TP53* were also reported in families who do not fulfil the classical criteria of Li-Fraumeni Syndrome[59]. These families show older ages of onset of breast cancer[60], suggesting lower penetrance of at least some *TP53* pathogenic variants. This is consistent with recent estimates of the prevalence of pathogenic germline *TP53* variants in the general population[61], which are also much higher than expected on the basis of the prevalence of Li-Fraumeni Syndrome alone. Furthermore, although with a large confidence interval, Dorling et al. found an OR of approximately 3 for TP53 truncating and missense variants, which is lower than initially demonstrated[58]. The other problem is the rarity of variants, which necessitates the analysis of very large case-control series in order to sufficiently narrow down confidence intervals of risk estimates. For this reason, we have reasonably good breast cancer risk estimates for the 1100delC variant in *CHEK2*, which occurs in ~0.5% of the general population in Europe[62, 63] and the USA[63, 64], but not for most other, much rarer variation in this gene. Even in the recently performed large gene panel studies[46, 47], wide confidence intervals of the

risk are often found. To establish an odds ratio of 2 with a 95% confidence interval of 1.4-2.8, conferred by a variant with an allele frequency of 0.01%, would require genotyping 100,000 cases and 100,000 controls. Larger numbers are needed for lower risks and lower allele frequencies. One way around this problem is to perform burden-type association studies, in which different variants are lumped together on the assumption that their impact on protein function is identical. This is an accepted approach for protein-truncating variants[46, 47], but is problematic for missense changes.

Gene panel studies – non-*BRCA1/2* genes

Gene panel sequencing (GPS) has become a diagnostic reality in cancer genetics. Due to the lower costs and improving data quality, it became possible to test multiple genes in addition to *BRCA1* and *BRCA2* in a single assay, driven by a desire to explain familial clustering of breast cancer in more families and thus impact clinical management. As explained above, the frequency of pathogenic variants found in clinic-based series of familial cases is dependent on the selection criteria of the families included. The highest frequencies, up to 10%, of pathogenic variants are still found in the *BRCA1* and *BRCA2* genes in familial breast cancer cases[65-67] compared to ~2.5% in population-based cases[46, 47]. Pathogenic variants in non-*BRCA1/2* genes are found in 3.7-6.2% of the familial cases[64-69]. The highest frequencies of pathogenic variants in non-*BRCA1/2* genes are found in *CHEK2, ATM* and *PALB2*[64]. However, this increased diagnostic yield comes at the expense of a large proportion of detected VUS, which poses a significant clinical problem. Gene panel studies have found a VUS in 13.6-41.6% of the cases[65, 67, 68, 70]. This means that for every pathogenic variant found in a case, 2 to 3 cases with a VUS are detected. Furthermore, gene panels may contain many genes for which the relevance to breast cancer is unknown or uncertain, as outlined above. Due to these uncertainties, most test-results of commercial gene panels do not translate well into cancer risk assessment. Even the relatively well-defined cancer risks conferred by *BRCA1* and *BRCA2* are influenced by mutation position and mutation class, as well as by other genetic factors, non-genetic exposures, and lifestyle factors[52, 71-73]. Therefore, the gain in clinical utility of testing genes for which evidence of their association with breast cancer is still ill-defined, remains limited[43, 74].

Common low risk variants and Polygenic Risk Scores

Since 2005, Genome-Wide Association Studies (GWAS), using SNP arrays and very large case-control samples, have enabled the identification of common low risk variants for breast cancer[25]. Collaborative groups such as the Breast cancer Association Consortium (BCAC), have identified ~180 common low risk variants associated with breast at genome wide significance level ($1x10^{-8}$)[23]. The first substantial batch of variants was found by the Collaborative Oncologic Gene environment Study (COGS) in 2013, coordinated by BCAC, which was subsequently confirmed and extended by combining with other GWAS data[75]. Another 65 loci were detected after the introduction of the OncoArray, a SNP array with

a much denser SNP coverage than COGS[23]. Some of the associated variants are more strongly associated with ER-negative or ER-positive subtypes of breast cancer[23, 76]. These initially 180 known associated variants explain 18% of the familial relative risk for breast cancer, but a much greater proportion (~40%) can be explained when variants that can be reliably imputed from the OncoArray data were included[23, 26]. Because many of these are expected to be relatively rare (<5%) and/or of very small effect-sizes, very large case-control studies are needed to reach genome-wide significance levels of association. More recent large pooled GWAS discovered already 38 novel breast cancer susceptibility loci at genome wide significance level[77, 78], although some of these loci are only associated with certain breast cancer subtypes.

The breast cancer associated common low risk variant alleles are distributed normally throughout the general population. This means that, in contrast to pathogenic variants in breast cancer susceptibility genes, all individuals in the population carry a certain number of risk alleles, with most individuals carrying the average number. Individually, these risk alleles confer a very small increase in breast cancer risk, but their joint effect may be a substantially higher[22]. In the absence of evidence of clear interactions between variants[22, 79], a simple log-additive (or multiplicative) model combines all variants into a single Polygenic Risk Score (PRS).

Many different PRS for breast cancer have been published in recent years. As published previously[2], Table 1 presents the effect sizes of published PRS until January 2019. Most studies presented here have generated PRS for overall unilateral breast cancer[22, 26, 80-89], few have addressed ER-status-specific PRS-models with the use of subtype-specific odds ratios of certain SNPs[26, 90]. Subtype-specific PRS can potentially be useful to guide clinical management for chemoprevention and other prevention strategies. Some studies[91-93] have used a PRS to predict contralateral breast cancer, and others studied the PRS as risk modifier in rare gene mutation carriers (*BRCA1*, *BRCA2, CHEK2*)[94-96]. The number of common low risk variants, their allele frequencies and effect-sizes determine the discriminatory and predictive power of a PRS. Predictive power of a PRS is usually expressed as Odds or Hazard Ratio (OR, HR) per Standard Deviation (SD) unit of the distribution; discriminatory power is often assessed by the area under the curve (AUC). The number of variants included in a PRS is not strongly correlated with the overall effect-size or the AUC. This is because the variants detected in the earliest studies, although smaller in number, generally have higher effect-sizes than those detected more recently in larger studies with more statistical power. Including large numbers of variants at lower than genome-wide significance thresholds may increase predictive power of the PRS, but at the expense of being less specific[26].

Table 1: Effect size and AUC of Polygenic Risk Scores

Study[a]		Cases	Controls	Variants	Effect size				AUC
					Measurement	Overall BC	ER-positive BC	ER-negative BC	
	Unilateral breast cancer								
McCarthy et al. 2015[80]	prospective cohort; women referred for breast biopsy	74	390	12	per SD	OR=2.30[1.06-4.99] P=0.035			0.685 [0.620-0.750] (including age)
Dite et al. 2015[81]	population based case-control; women <50yr	750	405	77	per SD, adjusted for age group	OR=1.46 [1.29-1.64], 2*10-16			0.61[0.58-0.65]
Mavaddat et al. 2015[22]	case-control study	33673	33381	77	per SD	OR=1.55 [1.52-1.58]	OR=1.63[1.60-1.67]	OR=1.45 [1.40-1.49]	0.622 [0.619-0.627]
Naslund-koch et al. 2016[82]	Danish population study	1301	19010	72	5th in comparison to 1st quintile	HR=1.82[1.53-2.18]			NA
Li et al. 2016[89]	Prospective cohort; breast cancer families	1496	2869	24	per SD	HR=1.38[1.22-1.56] P=2.9*10^-7			0.59[0.55-0.63]
Shieh et al. 2016[83]	nested case-control study; mammography screening cohort	486	495	83	4th quartile in comparison to 1st quartile	OR= 2.54[1.69-3.82], P<0.001			0.60 [0.57-0.64]
Muranen et al. 2016[84]	case-control study	1689	1269	75	per SD	OR=1.56 [1.45-1.68] P=9.2E-31			NA
Muranen et al. 2016[84]	Breast cancer families	181	1269	75	per SD	OR=1.82 [1.55-2.13] P=1.8E-13			NA
Maas et al. 2016[85]	prospective cohort; nested case-control study	17171	19862	24; 92	10th decile in comparison to 1th decile	OR=2.79 (24 variants)			0.623 (92 variants)
Cuzick et al. 2017[101]	prospective nested case-control study	359	636	88	Interquartile range	OR=1.37 [1.16-1.79] ; p-value <0.001	OR=1.44 [1.16-1.79] P<0.001	OR=0.99 [0.61-1.61] P=0.10	0.55 [0.51-0.60]
Shieh et al. 2017[90]	nested case-control study; mammography screening cohort	110 (ER-positive)	214	83	per SD		OR=1.58 [1.06-2.36] p=0.02		0.68 [0.61-0.75] p=0.07
Evans et al. 2017[86]	Case-control study; women attending a familial risk clinic	364 (112 *BRCA1/2*+)	1605 (691 *BRCA1/2*+)	18	Interquartile range	Non-carriers: OR=1.55 [1.29-1.87]			Non-carriers: 0.59[0.55-0.63]

Study[a]		Cases	Controls	Variants	Effect size				AUC
					Measurement	Overall BC	ER-positive BC	ER-negative BC	
van Veen et al. 2018[102]	Prospective cohort study	466	8897	18	interquartile range	OR=1.56 [1.38-1.77]			NA
Zhang et al. 2018[87]	nested case-control study	4006	7874	67	4th quartile in comparison to 1st quartile	RR=2.5 [2.2-2.8]			NA
Khera et al. 2018[88]	Case-control study; UK biobank participants	6586	157895	5218	5th quintile in comparison with remainder	OR=2.07 [1.97-2.19], P=3.4*10-159			0.69[0.68-0.69], including age
Mavaddat et al. 2019[26]	Prospective case-control studies	11428	18323	313	per SD	OR= 1.61[1.57-1.65]	OR=1.45 [1.37-1.53]	OR=1.35 [1.27-1.43]	0.630 [0.628-0.651]
Mavaddat et al. 2019[26]	Case-control study; UK biobank	3215	186825	306	per SD	HR=1.59 [1.54-1.64]			
	Unilateral breast cancer in gene mutation carriers								
Muranen et al. 2017[95]	*CHEK2* c.1100delC carriers	39,139 (624 carriers)	40,063 (224 carriers)	74	per SD	carriers: OR=1.59[1.21-2.09], P=0.0008; non-carriers: OR=1.58[1.55-1.62], P<1.0E-10			NA
Kuchenbaecker et al. 2017[94]	*BRCA1* mutation carriers	7,797	7,454	88	per SD	HR=1.14 [1.11-1.17], P=1.8*10-18	HR=1.11 [1.08-1.15], P=3.5*10-13	HR=1.27 [1.23-1.31], P=8.2*10-53	Overall BC: 0.541 [0.530-0.551]
Kuchenbaecker et al. 2017[94]	*BRCA2* mutation carriers	4,330	3,881	88	per SD	HR=1.22 [1.17-1.28], P=7.2*10-20	HR=1.22 [1.16-1.27], P=4.0*10-19	HR=1.15 [1.10-1.20], P=6.8*10-10	Overall BC: 0.566 [0.551-0.581]
	Contralateral breast cancer								
Sawyer et al. 2012[92]	Case-control study; Familial breast cancer cohort	126 contralateral BC	711 unilateral BC	22	4th quartile in comparison with 1st quartile	OR=1.96 [1.17-3.70]			NA
Robson et al. 2017[91]	Population based case-control study, <55yr	1,459 contralateral BC	2,126 unilateral BC	67	4th quartile in comparison with 1st quartile	OR = 1.75 [1.41-2.18]			NA

[a]For unilateral breast cancer, studies are added up to January 2019.

Abbreviations: AUC, Area Under the Curve; BC, Breast Cancer; ER, Estrogen Receptor; SD, Standard Deviation.

For all PRS-models predicting breast cancer, the AUC is modest, i.e., 0.6 – 0.7, but should this alone preclude their application as an individual test to predict if a woman will develop breast cancer or not? A comparison with gene panel testing, which is widely used in the clinic for this purpose, is illustrative. A PRS has been shown to be capable of stratifying women into different risk categories in a clinically meaningful way[22, 89, 92, 94], but the most relevant clinical information of the PRS is in the extreme tails of the distribution. Because these tails concern the general population (as opposed to gene mutation carriers only), the associated attributable risks of the PRS are in fact far greater than that achieved by gene panel testing. For example, the best performing PRS at this moment includes 313 common low risk variants (PRS_{313}) with an association at a p-value threshold two orders below genome-wide significance ($P<10^{-5}$). For this PRS, in the general population, 35% of all breast cancers occur in women in the highest quintile and only 9% of all breast cancers in the lowest quintile[26]. Women in the top 1% of the PRS_{313} are at 4-fold elevated risk relative to population average (95% CI 3.34-4.89), a risk-level defined in many countries as 'high'. In comparison, *BRCA1* mutation carriers explain <2% of all breast cancer in Western Caucasian populations[97] and comprise ~0.1% of the general population. Additional studies have shown that the PRS based on 313 variants is associated with both contralateral breast cancer in the population[98] and unilateral breast cancer among *BRCA1/2* gene mutation carriers[73]. Implementation research is ongoing to introduce the PRS into clinical genetic testing e.g. in the Netherlands, Germany, France, UK and USA. An example of how individual PRS-testing could aid risk counselling in the setting of familial breast cancer is shown in Figure 3, which highlights how two individuals that would otherwise have received the same risk assessment (sisters in generation IV) on the basis of their identical family history, are clearly classified into distinct risk classes on the basis of their PRS_{313}.

Another potential application of the PRS is in deciding when and how frequent women should undergo breast cancer screening[20, 99]. In most countries running such screening programs, women are offered screening above a certain age, usually between 45 and 50, when their breast cancer risk exceeds a certain cost-effective level. Women in the lowest quintile of the PRS_{313} in fact never reach that threshold, whereas those in the highest quintile will attain this level of risk before age 40 years[26]. A risk-based entry into population-screening, as opposed to the current age-based entry, could therefore be more cost-effective, although the evidence to support this notion has been derived only from modelling studies so far[20, 100].

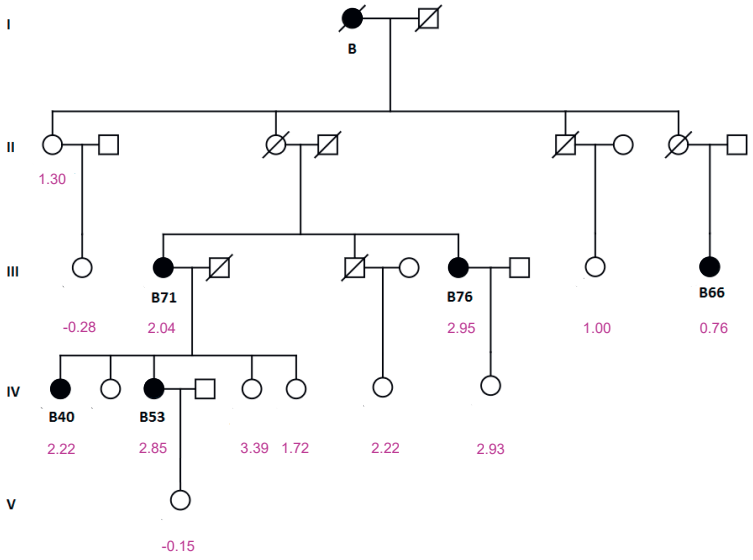

Figure 3. Standardised PRS for breast cancer cases and their female relatives
In this non-*BRCA1/2* breast cancer family, multiple family members were genotyped by SNP array. For all genotyped individuals, the SNP_{313} Polygenic Risk Score (PRS) was calculated. The individual PRS are standardised to population controls in the BCAC dataset (mean=0 and SD=1 in controls). The numbers in the figure are therefore Z-scores of the individual PRS. A higher Z-score indicates a higher breast cancer risk.

A limitation of many PRS is that most variants contained in it are discovered in European-descent populations and their effects cannot be translated directly to other ethnicities. Studies are ongoing to define breast cancer associated variants and evaluate the European-descent derived PRS in non-European populations. Recently, studies performed for the Asian population[103] and Latinas[104], showed similar performance for the PRS as in the European population, but for the African population[105] there was an attenuated effect size. Therefore, caution is needed when using the European-descent derived PRS for women of ancestries for which the effect of the PRS is dissimilar or not yet determined.

Hormonal, environmental and lifestyle risk factors

A number of non-genetic risk factors are presently firmly established as being associated with breast cancer. Besides age, these include physical factors such as body height and weight[106, 107]. For weight, breast cancer risk is dependent on menopausal status. Weight gain and obesity (BMI>30) after menopause are associated with an increase in postmenopausal breast cancer[106]. It is likely that higher oestrogen levels underlie this effect in postmenopausal women[108]. A higher mammographic density due to a high proportion of connective and glandular relative to adipose tissue, leads to a higher risk for breast cancer[27, 28, 109]. Hormonal factors influencing breast cancer risk include the use of oral contraception and hormone replacement therapy (HRT)[110, 111], as well as age at menarche and menopause[112]. Reproductive history (age of first childbirth or nulliparity) may have similar impact on mammary gland biology[28, 113]. The lifestyle factors alcohol use and smoking increase breast cancer risk as well, while physical activity and breastfeeding seems to act protectively[114-116]. Finally, a personal history of benign breast disease also signifies an increased breast cancer risk[28].

Combining risk factors

Since any woman will have only a single certain risk-level at a given moment in time to develop breast cancer over the course of her life, genetic and non-genetic risk factors must somehow combine to define that risk. A major challenge for individual breast cancer risk prediction, therefore, is to design risk calculation models that accommodate all known risk factors, which requires knowledge about the underlying model how they interact. Through the large international consortia such as BCAC, data to design and validate such models are now forthcoming. There are now much more accurate estimates how the PRS can modify the breast cancer risks conferred by pathogenic variants in *BRCA1*, *BRCA2* and *CHEK2*[73, 94, 95, 117, 118]. This can help inform choices and timing of preventive surgery or chemoprevention. The interaction between the c.1100delC variant in *CHEK2* and the PRS appears to follow a simple multiplicative interaction, but the per SD hazard ratio estimates in *BRCA1* and *BRCA2* pathogenic variant carriers were smaller than those in general population[73]. In *BRCA1* pathogenic variant carriers, the ER-negative PRS showed a much stronger association with breast cancer risk in comparison with the ER-positive

PRS, consistent with the predominant ER-negative tumour subtype in *BRCA1* pathogenic variant carriers[73, 94]. Few studies have been performed on *ATM* and *PALB2* pathogenic variant carriers, but a recent study showed that the effect sizes of the associations were in between those for *BRCA1/2* and *CHEK2*[119]. These issues highlight the complexity of some of these interactions and underscore the necessity of large prospective cohort studies to validate these models. A similar deviation from simple multiplicative interactions has been found for individuals with rare pathogenic variants in more than one breast cancer associated gene[120]. There is limited evidence for interaction between common low risk variants and lifestyle/hormonal factors[121]. Recent studies showed that the effect of these risk factors and the PRS can in general be combined in a multiplicative way[122, 123].

Breast cancer risk prediction models

Currently, predicting whether a healthy woman will develop a primary breast cancer or not is mainly done within clinical genetic services. Women who are worried because of their family history for breast cancer can be referred by their general practitioner to such a clinic; alternatively, breast cancer patients with a clear family history are referred by oncologists or surgeons, also because of the potential impact a gene diagnosis may have for their therapeutic options[124]. At the moment, the major incentive behind these referrals is the possibility to detect a high- or moderate risk variant in one of the breast cancer genes (i.e. *BRCA1*, *BRCA2*, *PALB2, CHEK2, or ATM)*. As set forth above, however, such variants are found in ~10% of all referred families. For women from breast cancer families where no pathogenic variant is found, clinical management is determined based on their lifetime breast cancer risk. The Dutch screening guideline (Table 2) advises women with a risk above 20% based on their family history to perform annual mammography from age 40, and to continue biennial screening at age 50 as part of the national population screening program. An intensified protocol has been designed for women with a risk >30%. Breast cancer risk prediction for healthy relatives is often based on family history alone, although more than 20 risk prediction algorithms known today[125] include other risk factors as well. Several studies have shown an improved discriminative power between breast cancer cases and controls by combining the PRS with a breast cancer risk prediction tool[81, 83, 101, 102]. In one study[89], new breast cancer lifetime risks for women from breast cancer families were calculated by adding the PRS to family-based risk prediction. For up to 23% of the women, screening recommendations, as stipulated by local management guidelines, could alter.

Some well-known risk prediction algorithms are the Gail model, BRCAPRO, Tyrer-Cuzick and the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA). Depending on what the model predicts and for which population, the most appropriate model can be used. The Gail model predicts breast cancer lifetime risks for women older than 35 years and is widely studied and validated. It includes hormonal risk

factors, breast biopsies and affected first degree relatives[125-127]. The Chen model extends this by incorporating mammographic breast density as well[128]. The BRCAPRO model calculates breast cancer lifetime risks and the risk of contralateral breast cancer. The estimation is based on family history (first- and second-degree relatives), the prevalence of *BRCA1* and *BRCA2* pathogenic variants, population incidence rates and pathological markers for breast cancers[127, 129]. The Tyrer-Cuzick model incorporates hereditary (first- and second-degree relatives with breast or ovarian cancer), hormonal and environmental risk factors (age, BMI, menarche, reproductive factors, menopause, and HRT) and pathological variables (breast biopsies and benign breast pathology)[125, 127]. Mammographic density and PRS were recently incorporated in the model[127]. BOADICEA estimates breast cancer lifetime risks and contralateral cancer risks for women with a family history of breast cancer[130]. The model includes tumour pathology characteristics, recent cancer incidences and pathogenic variants in *ATM, BRCA1, BRCA2, CHEK2,* and *PALB2*[131]. For BOADICEA, the family history is not restricted to a number of relatives or a particular degree. The current version, model V5, has been extended to accommodate a broad range of genetic and non-genetic risk factors for breast cancer, adding mammographic density, reproductive factors, age at menarche and menopause, use of hormones, BMI, body height, alcohol use and 4 different PRS including the PRS_{313} to the previous version[132]. In the new version, V6, available in February 2022, breast and ovarian cancer population incidences of the Netherlands will be added. Unsurprisingly, the potential for risk stratification was the greatest when all risk factors were used for risk prediction. Of all factors, the PRS had the largest contribution in risk stratification. Without knowledge of the genetic status of a woman for the rare genes, or family history, the lifetime breast cancer risk varied from 2.8% for the lowest, to 30.6% for the highest percentile of the PRS[132]. The model assumes that the risk factors and the PRS_{313} act multiplicatively, consistent with evidence from previous studies[123]. Similarly, the assumption that the PRS_{313} combines multiplicatively with the effects of rare truncating variants in the five breast cancer genes will need validation. Finally, the current BOADICEA model uses population breast cancer risks of several countries but UK risk factor distributions and therefore may require tailoring for application in other populations. The BOADICEA model is incorporated in the user-friendly web interface CanRisk[133] and externally validated[134]. Within clinical genetic services of the Netherlands, CanRisk is already used by some clinicians for risk prediction in families where no pathogenic variant is found, but currently mostly only family history is included as variable.

Outline of this thesis

The main objective of work presented in this thesis was to explore the clinical utility of the Polygenic Risk Score (PRS) based on breast cancer associated common low risk variants for individual breast cancer risk prediction. It did so by generating knowledge about the PRS in the Dutch general population and in clinic-based breast cancer families, as well

as in a large international population of *BRCA1/2* pathogenic variant carriers. The results will support implementation of comprehensive risk prediction by using CanRisk in the clinic, and may help women to make more informed choices about their optimal clinical management.

Table 2: Dutch screening guideline

	Low (RR: <2)	Moderate (RR: 2-3)	High (RR: >3)
Lifetime risk	<20%	20-30%	>30%
Start screening	50 yr	40 yr	35 yr
Mammography	Population screening	<50 yr annual >50 yr population screening	<60 yr annual >60 yr population screening
MRI	-	-	-

Chapter 2 explores the clinical applicability of a 161-variant-based PRS for risk prediction in a cohort of 101 high-risk breast cancer families not explained by pathogenic variants in the *BRCA1* and *BRCA2* genes. The association with breast cancer and the clinical impact of the PRS on risk prediction was investigated for affected and healthy women from these families by determining the potential change in clinical management.

Chapter 3 explores the clinical applicability of the 313-variant-based PRS for risk prediction in a cohort of almost 4,000 familial Dutch breast cancer cases who tested negative for pathogenic variants in *BRCA1/2* and of whom the majority were evaluated in research setting for pathogenic variants in *PALB2, CHEK2*, and *ATM*. The clinical impact of addition of the PRS on breast cancer risk prediction by BOADICEA based on family history and pathogenic variant carrier status was investigated by determining the potential change in clinical management. In Appendix 1, this study is used as illustration to discuss the situation with regard to the review by the Medical Ethical Committees of multicentre research in the Netherlands that is not covered by the Dutch medical research involving human subjects act (wet medisch-wetenschappelijk onderzoek met mensen, WMO) [article in Dutch].

Chapter 4 assesses the clinical validity of the 313-variant-based PRS by determining the association between this PRS and breast cancer in the Dutch population. Furthermore, we validated the risk prediction algorithm BOADICEA by exploring the discriminative ability of an individualised 10-year breast cancer risk score based on the PRS and several known risk factors. We also assessed how a risk-based approach of population-based screening could have impacted breast cancer detection rates in our study cohort.

In chapter 5, we investigated whether the 313-variant-based PRS for breast cancer is associated with contralateral breast cancer risk among women with pathogenic variants in *BRCA1 or BRCA2* and explored the implications for contralateral breast cancer risk prediction for these women.

In chapter 6, we summarised the results of our pilot study, the Individualised Breast cancer Risk prediction (IBR) study in which we included unaffected women from breast cancer families where no pathogenic variant is found. The aim of this study was to establish the percentages of women shifting to another risk category with comprehensive risk prediction (CRP) calculated using CanRisk, based on family history, the PRS_{313} and lifestyle/hormonal risk factors compared to the current family history-based risk prediction. Furthermore, the psychosocial impact of this new CRP will be assessed and described by Bredart et al. (*manuscript submitted*).

In chapter 7 we conclude with a general discussion about our main findings and future perspectives for implementation of CRP for breast cancer in the clinic.

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