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From *BRCA1* to Polygenic Risk Scores: Mutation-Associated Risks in Breast Cancer-Related Genes

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Abstract
Background: There has been huge progress over the last 30 years in identifying the familial component of breast cancer. ***Summary:*** Currently around 20% is explained by the high-risk genes *BRCA1* and *BRCA2*, a further 2% by other high-penetrance genes, and around 5% by the moderate risk genes *ATM* and *CHEK2*. In contrast, the more than 300 low-penetrance single-nucleotide polymorphisms (SNP) now account for around 28% and they are predicted to account for most of the remaining 45% yet to be found. Even for high-risk genes which confer a 40–90% risk of breast cancer, these SNP can substantially affect the level of breast cancer risk. Indeed, the strength of family history and hormonal and reproductive factors is very important in assessing risk even for a BRCA carrier. The risks of contralateral breast cancer are also affected by SNP as well as by the presence of high or moderate risk genes. Genetic testing using gene panels is now commonplace. ***Key-Messages:*** There is a need for a more parsimonious approach to panels only testing those genes with a definite 2-fold increased risk and only testing those genes with challenging management implications, such as *CDH1* and *TP53*, when there is strong clinical indication to do so. Testing of SNP alongside genes is likely to provide a more accurate risk assessment.

Introduction

The inherited aggregation of breast cancer has been intimated for over 130 years [1]. A family history of breast cancer in a first-degree relative has been linked to an approximate 2-fold relative risk [2]. Approximately 4–5% of all breast cancers are thought to result from inheriting high-risk dominantly inherited pathogenic variants (PV) [2] (but only 2–3% result from *BRCA1/2* mutations). However, around 27% are thought to have some form of inherited component, as reported in twin studies [3]. This means that the majority of the inherited components of breast cancer are likely due to polygenic inheritance rather than inheritance of a single gene PV.

Relatively few women with breast cancer present with a clear pattern of cancers in the family consistent with that reported by Broca [1]. However, clusters of breast cancers within families, particularly occurring at younger ages, are not infrequent and account for approximately 5% of cases overall [3]. As even genes conferring the highest risk do not cause breast cancer in every woman in their lifetime unaffected carriers of the "cancer gene" may mask true inherited families and chance clusters may mimic inherited disease.

In many instances of familial breast cancer, there is a high incidence of other tumors, notably ovarian, prostate, and pancreatic cancer or in rarer instances sarcomas and brain tumors. Empiric risks for women who have particular types of family history have been calculated [2]

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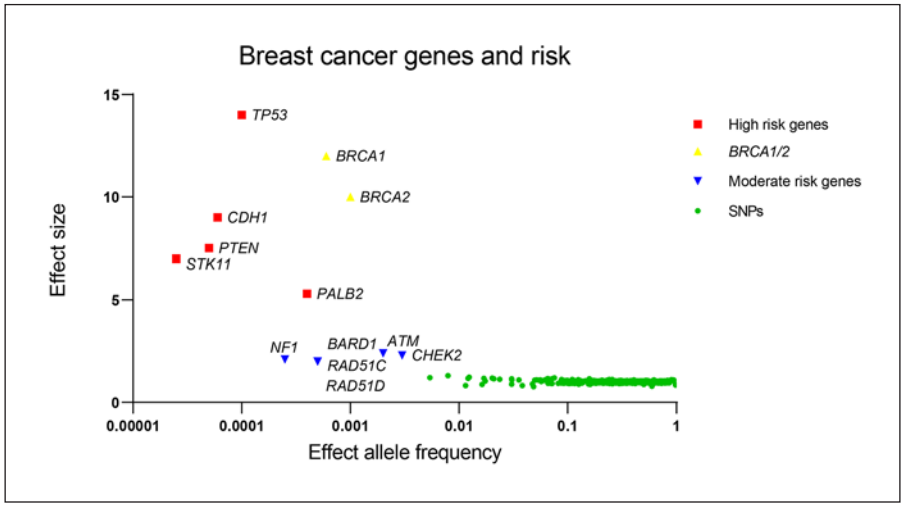

Fig. 1. Relative risk and allele frequencies of high-, moderate-, and low-risk genetic variants.

and have led to criteria for proposed enrolment in familial breast cancer clinics. However, increasingly risk is informed by undertaking panel tests of genes linked to breast cancer risk and also tests for multiple common variants in a polygenic risk score (PRS).

This paper will cover all of the known genes and genetic variants linked to breast cancer risk from the discovery of the first bona fide breast cancer genes including *BRCA1* through to the development of PRS from multiple single-nucleotide polymorphisms (SNP) discovered through genome-wide association studies.

Generally, genes and SNP have been divided into high-risk genes with an OR ≥4.0 (lifetime risks in European populations ≥40%), moderate risk genes with an OR ≥2.0 but < 4.0 (lifetime risks in European populations ≥20% but < 40%), and genetic modifiers with an OR of 1.01–1.5 (Fig. 1).

Molecular Genetics

High-Risk Breast Cancer Genes

BRCA1

Molecular geneticists started to try to identify breast cancer genes in the mid 1980s. By 1990 the first gene (*BRCA1*) had been identified on chromosome 17q by linkage analysis in breast cancer families [4]. It quickly became apparent that the locus later termed *BRCA1* conferred a risk of both breast cancer and ovarian cancer [5], as originally predicted by Henry Lynch and Krush [6] some 20 years earlier. The gene itself was cloned in 1994 [7]. The *BRCA1* gene is very large, with a 7,207-bp transcript. It has only limited homology with any previously identified human sequence, and its function is not fully clarified. The main functions are homologous repair of double-stranded DNA breaks and transcriptional activation. *BRCA1* is predominantly a breast/ovarian cancer gene conferring lifetime risks of 50–85% for breast cancer and 30–60% for ovarian cancer [8–11]. Initial assessments based on high-risk families tended to provide risk estimates only appropriate for that setting [8, 9]. However, attempts to strip out the biases of familial ascertainment providing very low estimates were not really realistic in relation to the real situation outside of those identified with no family history of breast or ovarian cancer [12]. The best current estimates are based on prospective studies [10, 11], which lack the ascertainment bias of the earlier historic studies. Nonetheless, clinicians and genetic counsellors should refrain from providing a very specific risk (e.g., 72% breast cancer risk by age 80 years for *BRCA1*) [11] as the risks will vary with nongenetic risk factors such as reproductive factors as well as the degree of family history. These factors can be incorporated into a model developed from the BOADICEA algorithm called CanRisk [13]. The addition of a PRS from SNP (see below) is likely to provide an even more accurate likelihood of the breast and ovarian cancer risk, with overall likelihoods varying from as little as 45% to > 95% for female breast cancer in *BRCA1* [14]. The risks of estrogen receptor negative (ER–) breast cancer varied from 59 to 83% at the 5th and 95th percentiles, while the risks of ovarian cancer by age 80 years were 30 and 59% for *BRCA1*. The pathology of *BRCA1*-related cancers is fairly specific, with the great majority of ovarian cancers being high grade serous and around 70% of breast cancers being ductal triple negative (negative for HER2, ER, and progesterone receptor) [15]. There is no evidence of an increased risk of mucinous ovarian cancer [15, 16] and this should be discounted in algorithms to predict the likelihood of *BRCA1*, as it is in the pathology-adjusted scoring system [17]. Similarly, HER2 positivity is uncommon in *BRCA1* PV carriers and should reduce the likelihood of finding a causative variant [17]. There is no strong convincing evidence for the risk of other cancers in *BRCA1* carriers [18].

A number of other cancers have been shown to potentially be related, including early-onset prostate cancer [19, 20], colorectal cancer [19, 21], endometrial cancer [19, 22], and pancreatic cancer [20]. However, the OR for these cancers are generally < 3-fold and have not been convincingly replicated [18, 23, 24]. Although there may be a specific risk of high-grade serous endometrial cancer as this translates to < 10% of all endometrial cancers, this is unlikely to translate into a substantial OR for endometrial cancer as a whole [24]. Furthermore, a study comparing *BRCA1* and *BRCA2* found that it was *BRCA2* that harbored the truly more heterogeneous cancer risk [25]. Indeed, the only prospective study of early detection outside of breast and ovarian cancer showed no evidence of benefit for prostate cancer in *BRCA1*, with cancer incidence not being significantly increased compared to controls [26]. Overall, *BRCA1* carriers should be advised that there is unconvincing evidence of substantial increased risks of cancers beyond breast and ovarian cancer and that if there is any increased risk it is unlikely to be sufficient to warrant early detection measures.

BRCA2

Soon after the *BRCA1* locus was identified, it became clear that many large breast cancer kindreds, particularly those with an affected male, were not accounted for by *BRCA1*. A second locus, i.e., *BRCA2*, was mapped by family linkage analysis to chromosome 13q in 1994 and within a year the gene had been isolated [27]. The size of the gene is even greater than that of *BRCA1* (11,386 bp), with which it shows some homology particularly with regard to homologous repair and cancer predisposition. *BRCA2* PV confer female lifetime risks of 40–87% for breast cancer and 10–30% for ovarian cancer [8–11, 28]. The effect of a breast cancer family history on breast cancer risk is even greater for women with *BRCA2* PV [8–11, 28]. Estimates of the *BRCA2* breast cancer risk have been as low as 38%, stripping out any additional familial risk effects [12]. However, these studies, which rely on historic data, include women born before 1930 in whom breast cancer risks are much lower than those for modern day women [28]. Again, the best current risk estimates are based on prospective studies [10, 11]. Like for *BRCA1*, clinicians and genetic counsellors should refrain from providing a very specific risk, such as 69% breast cancer risk by age 80 years for *BRCA2* [11], as similarly to *BRCA1* the risks will vary with nongenetic risk factors such as reproductive factors as well as the degree of family history. These factors can be incorporated into a model developed from the BOADICEA algorithm called CanRisk to give a personalized risk assessment based on germline genetic and other known risk factors [13]. The addition of a PRS from SNP (see below) is likely to provide an even more accurate likelihood of breast and ovarian cancer risks, with overall likelihoods varying from as little as 43% to > 95% for female breast cancer in *BRCA2* [14]. The risks breast cancer varied from 57–81% at the 5th and 95th percentile, but they were as little as 43% with no family history and as high as 85% with a family history of breast cancer. Likewise, the ovarian cancer risks by age 80 years at the 5th and 95th percentile were 10 and 28% for *BRCA2*. The pathology of *BRCA2*-related breast cancer is not as specific as that for *BRCA1*, although there is a trend toward higher-grade ductal ER+ PR+ HER2–, with only 16% of cases being triple negative, but unlike *BRCA1* the likelihood of triple-negative increases with age [15]. HER2 positivity is less common but not as infrequent as for *BRCA1* PV carriers [17]. The great majority of ovarian cancers are high-grade serous [15]. Similarly to *BRCA1*, there is no evidence of an increased risk of mucinous ovarian cancer [15, 16]. Males have a substantially increased risk of breast cancer, with a lifetime risks of 5–14% [29, 30]. One study using a PRS found that the risk of breast cancer by age 80 years is 5% for men at the 5th percentile of the PRS and 14% for men at the 95th percentile [30]. In addition to breast and ovarian cancer risk, *BRCA2* PV clearly also predispose to prostate cancer (OR = 2.5–6.3), pancreatic cancer (OR = 3.5–5.9), gastric cancer (OR = 2.4–2.59), and various skin cancers including melanoma, all of which have been validated in at least 2 studies [18, 31–33]. Though rare, ocular (uveal) melanoma appears to also be strongly linked [18, 34]. As above, a study comparing *BRCA1* and *BRCA2* found that *BRCA2* conferred the greater cancer risk beyond breast and ovarian cancer [25]. The prospective IMPACT study of early detection of prostate cancer showed an excess risk in *BRCA2* carriers and that PSA was effective at earlier detection of the predominantly more aggressive higher Gleason score prostate cancer [26].

Li-Fraumeni Syndrome and TP53

Even before the identification of *BRCA1/2*, the *TP53* gene had been implicated in hereditary breast cancer as part of the Li-Fraumeni cancer family syndrome [35]. It was, however, recognized that this accounted for only a very small proportion of breast cancer families and subsequent studies confirmed this impression. Nonetheless, mutations in *TP53* may account for almost as many breast cancers in patients ≤30 years of age as *BRCA2* [36], and diagnosis at an age ≤30 years is a criterion for testing by the Chompret criteria [37]. Overall, 2–8% of breast cancers in patients aged ≤30 years harbor a *TP53* germline PV and these are more common with HER2+ invasive disease and high-grade comedo-DCIS [38]. Detection rates drop dramatically after 30 years of age, and testing of women after age 45 years with no previous malignancy and no other element of Chompret criteria fulfilled (no typical Li-Fraumeni cancer in a close relative) is not rec-

Table 1. Genes associated with a moderate or high lifetime risk of breast cancer and effects on life expectancy

Disease gene	Location	Tumors	Tumor age, years	Risk, %	Birth incidence of PV	Life expectancy[a]
High-risk genes						
BRCA1	17q	Breast (women)	>18	50–90	1 in 800	62 years
		Ovary	>35	30–60		
BRCA2	13q	Breast (women)	>18	40–90	1 in 4–800	68 years
		Ovary	>40	10–30		
		Prostate (men)	>30	25		
		Pancreas	>30	5		
LFS *TP53*[a]	17p	Sarcoma	1st	80	1 in 5,000	Severely reduced
		Breast cancer (women)	>16	80–95		
		Gliomas	1st	20		
PALB2	16	Breast cancer	>25	40–60	<1 in 1,000	Normal
		Ovarian cancer	>40	4–5		
		Pancreatic cancer	>40	2–3		
HDGC *CDH1*[a]	16q	Gastric	>16	70–80	Very rare	Reduced
		Breast	>20	40–80		
PTEN Cowden	10q	Breast cancer	>25	60	1 in 10,000–250,000	Reduced in women
		Thyroid	30	10		
STK11	19p	Gastrointestinal malignancy	20	60	1 in 25,000	58 years
		Breast	>30	30–50		
Moderate-risk genes						
CHEK2	22q	Breast cancer	>25	40	1 in 200	Normal
ATM	11q	Breast cancer	>25	20	1 in 300	Normal
RAD51D	17	Breast cancer	>25	20	1 in 1,000	Normal
		Ovary	>40	5–10		
RAD51C	17	Breast cancer	>25	20	1 in 1,000	Normal
		Ovary	>40	5–10		
NF1	17q	Neurofibroma	1st	100	1 in 2–3,000	54–72 years
		Glioma	1st	12		
		Breast cancer	>25	17		
BARD1	2	Breast cancer	>25	20	1 in 1,000	Normal

[a] Will include mortality from other cancers associated with PV of that cancer predisposition gene.

ommended [37]. This is due to the much likelier possibilities of identifying clonal hematopoiesis of indeterminant potential (CHIP) or a variant of uncertain significance, which could be misclassified as likely pathogenic erroneously [37]. People identified as carrying PV in *TP53* have a very high lifetime risk of malignancy, although this may vary with the exact variant, with dominant negative missense variants in the core binding domain conferring the highest risks [37]. There are particularly high risks of sarcoma, especially osteosarcoma and embryonal rhabdomyosarcoma, gliomas, and other brain malignancies such as choroid plexus carcinoma and SHH-medulloblastoma as well as adrenocortical carcinoma [37]. Although screening has been shown to have an impact, with whole-body MRI, breast MRI, and dedicated brain MRI now being recommended, the psychological impact of being identified as a *TP53* PV carrier or erroneously being misdiagnosed is considerable.

Cowden Syndrome and PTEN

The *PTEN* gene on chromosome 10q has been identified as the causal gene in Cowden syndrome, in which early-onset breast cancer is associated with a variety of other features including hamartomas of the skin and mucous membranes, thyroid adenomas and cancer, colonic polyps (including juvenile polyps), and craniomegaly [39]. While prospective breast cancer risk data is lacking due to its rarity, women with Cowden syndrome are at a high breast cancer risk and ought be offered equivalent high-risk breast cancer risk reduction measures. Addi-

tional tumor risks include thyroid cancer and endometrial cancer. Due to its rarity and usual syndromic features of marked macrocephaly, *PTEN* is rarely found on gene panels in the absence of diagnostic features (< 0.1%) [40].

Peutz-Jeghers Syndrome and STK11

STK11 (LKB1) is associated with the dominantly inherited condition Peutz-Jeghers syndrome (PJS), which is characterized by typical benign PJS polyps throughout the gastrointestinal tract and muco-cutaneous pigmentation (particularly on the lips). The breast cancer risk is probably between 40 and 60% lifelong [41]. Due to the rarity and distinct clinical features of PJS, *STK11* PV are extremely rarely found on gene panels (Table 1).

Hereditary Diffuse Gastric Cancer and CDH1

Mutations in the gene *CDH1* causes the dominantly inherited condition hereditary diffuse gastric cancer. Women with mutations in *CDH1* again have a 40–60% lifetime risk of breast cancer, often of lobular histology [42]. Due to its rarity and frequent family history of diffuse gastric cancer, *CDH1* is rarely found on gene panels (< 0.1%) [40], and some countries recommend that it be excluded from gene panels in the absence of familial lobular cancer or diffuse gastric cancer due to the consequences of erroneously identifying women as PV carriers [43].

PALB2

Upon first identification, *PALB2* was identified as a moderate-penetrance breast cancer susceptibility gene. In a case-control study, 10 truncating PV were identified in 923 individuals with familial breast cancer but no PV were identified in 1,084 healthy controls ($p = 0.0004$) [44]. Despite the absence in controls, the relative risk of breast cancer associated with a PV in *PALB2* was only assessed as moderate in this discovery paper with a 2-fold OR [44]. However, subsequent assessment of risk in families confirmed a high risk, with an OR of 7.18 (95% CI 5.82–8.85; $p = 6.5 \times 10^{-76}$), and a lifetime risk of around 50% [45]. As with *BRCA2* the risks vary based on the family history and presumably the SNP PRS profile [45]. There is likely a small increase in the ovarian cancer risk and the evidence for an increased risk of pancreatic cancer is also fairly robust [45].

Moderate-Risk Genes

Ataxia-Telangiectasia

Ataxia-telangiectasia (*ATM*) was the first moderate-penetrance breast cancer gene for which there was strong clinical evidence. The possibility that *ATM* could be a breast cancer susceptibility gene was first proposed nearly 40 years ago when epidemiologists suggested that relatives of patients with an autosomal recessive condition called ATM, which predisposes to cancer in childhood, particularly lymphoid cancers, had an increased risk of breast cancer. The role of *ATM* in breast cancer susceptibility has been investigated in many studies. The first conclusive study identified 12 mutations in 443 familial breast cancer cases and 2 in healthy controls ($p = 0.0047$), suggesting that the relative risk in female *ATM* mutation carriers is 2.37 [46]. A 2- to 3-fold OR for *ATM* has been confirmed in many studies, consistent with lifetime risks of 20–30% [47]. There is nonetheless evidence of one dominant negative missense variant in *ATM* c.7271T>G, i.e., p.(Val2424Gly), that is consistent with a 60% lifetime risk [48, 49]. However, the risks for other missense variants may be < 2-fold and these are difficult to classify [47, 50].

CHEK2

CHEK2 is a gene that encodes a cell cycle checkpoint protein kinase that phosphorylates *TP53* and *BRCA1* and is involved in DNA repair. The relative risk of breast cancer in carriers of the *CHEK2* c.1100delC allele was estimated to be 2.2 [51], and it appears to be similar for other truncating PV in *CHEK2* [47]. Missense variants can be associated with increased risks, but these are generally below 2-fold [47]. PRS appear to be helpful in more accurately defining risk in *CHEK2* PV carriers [52], and individual risks can be assessed taking into account reproductive and other risk factors as for *BRCA1/2* [13]. There are no clear additional risks to *CHEK2* PV carriers, although increased risks of colorectal and prostate cancer have been found in some studies.

Neurofibromatosis 1

Women with the inherited tumor-prone condition neurofibromatosis 1 (NF1) are now thought to be at a moderately increased risk of developing breast cancer [53–55]. There is a particularly high OR in patients aged < 50 years, with a 10% risk by that age, after which the risk levels off [54]. The breast tumors associated with *NF1* PV have adverse pathological features, with higher proportions of grade 3 ER– and HER2+ and poor survival [55, 56]. As NF1 is an easy syndromic diagnosis with clinically easily detectable features (café au lait patches, skin neurofibromas) in the vast majority of individuals gene panel testing is unlikely to be required and it is very unlikely that testing will find a PV in *NF1* without these features [57].

Each of these moderate penetrance genes makes a relatively small contribution to the overall familial risk of breast cancer. Compared to the 16–18% of familial risk accounted for by mutations in *BRCA1* and *BRCA2*, currently it is estimated that moderate-penetrance breast cancer susceptibility genes only account for 6–7% of the familial risk (Fig. 2). In keeping with findings in *BRCA1*

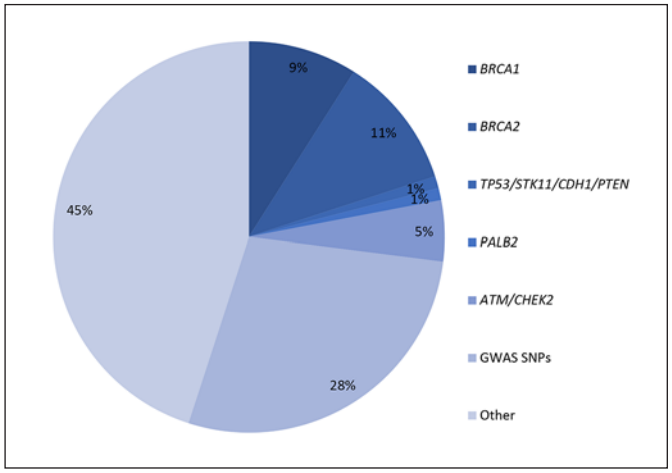

Fig. 2. Proportion of the familial component caused by known genetic factors. GWAS, genome-wide association study.

and *BRCA2*, most of the PV in these genes lead to premature protein truncation through nonsense codons or translational frameshifts. A very small number are possibly due to missense sequence variants. The moderate-penetrance breast cancer susceptibility genes each harbor multiple different rare PV.

Other Probable Moderate-Risk Genes

Three further genes that had previously been linked to breast cancer risk, but for which validation studies were inconclusive, were also supported in the BRIDGES study of 60,000 cases and 53,000 controls as having close to a 2-fold relative risk. These are the ovarian cancer genes *RAD51C* (OR = 1.93; 95% CI 1.20–3.11) and *RAD51D* (OR = 1.80; 95% CI 1.11–2.93) as well as *BARD1* (OR = 2.09; 95% CI 1.35–3.23). All 3 genes were particularly strongly linked to triple-negative breast cancer [47].

Genes Probably Spuriously Linked to Breast Cancer

BRIP1

In 2006 *BRIP1*, a BRCA1-interacting helicase (also known as BACH1), was also identified as a probable rare moderate-penetrance breast cancer susceptibility allele. In a case-control study, 9 mutations were identified in 1,212 familial breast cancer cases compared to 2 in 2,081 healthy controls ($p = 0.003$); the relative risk of breast cancer in monoallelic carriers of *BRIP1* mutations is 2.0 [58]. More recent work has, however, shown that the original link with breast cancer was spurious [59], and a large case-control study of over 60,000 cases showed an OR of only 1.11 with a 95% CI (i.e., 0.80–1.53) excluding a 2-fold risk [47]. At present, germline *BRIP1* mutations are considered a risk factor for postmenopausal ovarian cancer.

In addition to *BRIP1* a number of other genes identified as significantly linked to breast cancer have been shown by the BRIDGES study to be likely spurious [47]. These include *NBN* (OR = 0.90; 95% CI 0.67–1.20), *FANCM* (OR = 1.06; 95% CI 0.90–1.26), *RECQL* (OR = 0.84; 95% CI 0.64–1.10), *RAD50* (OR = 1.08; 95% CI 0.83–1.40), and *XRCC2* (OR = 0.96; 95% CI 0.47–1.93).

Missing Heritability of Breast Cancer Predisposition

While recent population-based studies have estimated the frequency of germline *BRCA1/2* PV to be as high as 1 in 200 [47, 60], outside of strong founder populations such as the Icelandic and Jewish, this is insufficient to account for more than 20% of the *heritable* component of breast cancer and only about 2% of all breast cancers [61, 62]. The discrepancy has become even greater with the recognition that many women with germline mutations in either *BRCA1* or *BRCA2* have an average lifetime risk of developing breast cancer ("penetrance") of around 65–70% or less rather than the 85–90% originally generated from high-risk families. While there may be families and individuals with hitherto undetected germline *BRCA1/2* mutations, or novel mechanisms of disruption, e.g., epigenetic silencing [63], structural variants, or deep intronic variants causing splicing that are missed by standard DNA testing, the evidence for these reducing sensitivity by more than 2–5% is limited [64]. It is nonetheless likely that the majority of the missing heritability is due to the presence of undiscovered low-penetrance genetic modifiers (Fig. 2).

Low-Risk Genetic Susceptibility

For longer than a decade genome-wide association studies have been performed in order to identify associations between common variants and disease. This has led to the robust identification of more than 300 SNP that are associated with breast cancer risk [65–83]. These common low-risk alleles only confer a small risk by themselves, but when combined in a PRS the SNP provide a more informative risk estimate. It has been estimated that these SNP currently explain 28% of the familial risk of breast cancer [83] (Fig. 2), though much of the remaining 45% of the familial component of breast cancer yet to be discovered is thought to be likely more SNPs.

Due to the increase in the number of SNP found to be associated with breast cancer, and the increasing number of patients included in these association studies, subgroup analysis has shown that there are several SNP that are more strongly associated with ER-negative disease than with ER-positive disease and vice versa [75, 76, 79, 83–87]. Similarly, it has been proposed that these SNP potentially can be utilized to modify the risk of PV carriers (see above) [88].

Table 2. Frequency of PV in panel tests with controls from the BRIDGES study

Gene	Breast cancer cases tested, *n*	PV, *n*	%	Controls in BRIDGES[b] (*n* = 50,706), %	OR	95% CI	*p* value
CHEK2[a]	62,692	1,668	2.66	0.62	4.39	3.89–4.95	<0.0001
BRCA2[a]	77,439	1,415	1.83	0.27	6.87	5.76–8.19	<0.0001
BRCA1[a]	77,439	1,350	1.74	0.11	16.05	12.28–20.97	<0.0001
ATM[a]	62,671	777	1.24	0.30	4.18	3.51–4.97	<0.0001
PALB2[a]	65,935	627	0.95	0.11	8.68	6.61–11.42	<0.0001
PMS2	35,737	117	0.33	0.07	4.76	3.28–6.92	<0.0001
BRIP1[a]	59,512	191	0.32	0.15	2.15	1.64–2.80	<0.0001
MSH6	35,737	109	0.31	0.05	6.20	4.03–9.53	<0.0001
TP53[a]	79,368	238	0.30	0.00	15.04	2.76–150.7	<0.0001
RAD50[a]	59,375	164	0.28	0.24	1.15	0.91–1.45	0.2594
BARD1[a]	59,375	158	0.27	0.06	4.51	3.05–6.66	<0.0001
RAD51C[a]	59,512	127	0.21	0.05	4.34	2.82–6.66	<0.0001
NBN[a]	59,375	120	0.20	0.20	1.02	0.78–1.32	0.9462
NF1[a]	56,097	90	0.16	0.03	5.43	3.14–9.38	<0.0001
MRE11A[a]	59,375	79	0.13	0.11	1.21	0.86–1.70	0.3307
RAD51D[a]	56,230	64	0.11	0.05	2.31	1.46–3.67	0.0003
MSH2	35,737	38	0.11	0.03	3.60	2.00–6.59	<0.0001
MLH1	35,737	35	0.10	0.02	4.97	2.46–10.27	<0.0001
PTEN[a]	79,157	71	0.09	0.01	9.10	3.68–22.55	<0.0001
CDH1[a]	77,273	53	0.07	0.02	3.48	1.77–6.84	<0.0001

[a] In breast cancer panels. [b] Only protein truncating variants, so will exclude CNV and missense variants which, for genes like *TP53, BRCA1, PMS2, MSH2, MLH1*, and *MSH6*, will underestimate carrier frequency and overestimate relative risk. We used 1 in 5,000 to account for this in *TP53* [38].

Numerous studies have validated the predictive power of these SNP, as well as their added value for existing prediction models based on classical risk factors [88–94].

The majority of the SNP related to breast cancer risk have been found and validated using data of studies participating in the Breast Cancer Association Consortium (BCAC), which is a collaboration involving over 100 international case control studies. The vast majority of studies included in the BCAC are performed in populations of European ancestry. Therefore, the current PRS is mostly applicable to women of European ancestry. With some adjustments, the current PRS may be suitable for women of Asian ancestry [95]. However, for women with any other ancestry, the current PRS provide no correct predictive estimate of the breast cancer risk.

Application of an SNP PRS alongside a risk evaluation tool such as Tyrer-Cuzick and a measurement of mammographic density can identify about 45% of breast cancers in the top 20% of the population [94].

Detection of Mutations in Known Breast Cancer Genes

While varied laboratory techniques were used previously to detect germline mutations of the known high-risk breast cancer genes, these were time consuming, had a limited sensitivity, and were offered to families in which there was a high likelihood of detection of a germline mutation. The introduction of Sanger sequencing to detect intragenic sequence variants followed by the addition of MLPA (multiplex ligation-dependent probe amplification) to detect whole exon or gene deletions (accounting for 10–15% of germline *BRCA1/2* [15–20% *BRCA1* and 4–5% *BRCA2*] PV) has resulted in a much higher mutation detection rate. In recent years, advances in genomic technologies with massively parallel sequencing approaches have lowered the costs further and increased the sensitivity of mutation detection, further enabling testing to be offered to a wider patient population.

With these technological advances comes a separate and new set of challenges including accurate classification of the variants identified [96]. Equally important is ensuring that, for mutations detected in the mainstream setting, at-risk family members are offered testing for the familial PV where appropriate [97]. Where specific founder PV are present in certain population groups, laboratories will often retain a Sanger sequencing-based specific assay for their detection. For example, as approximately 2–2.5% of Ashkenazi Jewish women carry 1 of 3 specific mutations (*BRCA1* c.68_69delAG, *BRCA1* c.5266dupC, or *BRCA2* c.5964delT), which collectively account for around 60% of all familial breast cancers in

this population group, testing for these alone will provide meaningful predictive information even when negative. For example, exclusion of the 3 PV in an Ashkenazi Jewish woman who has a family history of breast cancer will reduce her lifetime risk by 40–50%, as the 3 mutations make up 50% of the inherited risk. A similar calculation applies to Icelandic women, among whom the *BRCA2* c.771_775del p.(Asn257LysfsTer17) founder mutation accounts for a high proportion of breast cancer families [98]. Given the relatively low cost of full BRCA testing and of undertaking panels, testing for common PV is now much less commonly carried out unless these account for the great majority of hereditary high-penetrance variants in a country or population.

Breast Cancer Panels

Until around 2014 the great majority of breast cancer genetic testing was bespoke testing and suggested that *NBN* did qualify based on 1 protein-truncating variant, i.e., c.657del5, but BRIDGES did not confirm this for protein-truncating variants as a whole [47]. An idea of detection rates from commercial testing can be gained from testing of breast cancer cases with *BRCA1/2* alone, with targeted testing of *TP53* and syndromic genes such as *PTEN, STK11, NF1*, and *CDH1* when individual features or the family history indicated. Since that time testing has increasingly been carried out using next-generation sequencing panels of a much larger series of genes. Typically, commercial companies offer a breast cancer panel and wider panels that include genes that have often never been linked convincingly (see above spurious section). In 2015 Easton et al. [99] suggested limiting breast cancer panels to genes that at least demonstrated a 2-fold OR of breast cancer. At the time only *BRCA1*, *BRCA2, PALB2, ATM, CHEK2*, and *TP53* met the strict case-control qualifications, with syndromic genes only qualifying based on cohort studies. The article used the Ambry Genetics online tool (https://www.ambrygen.com/providers/resources/prevalence-tool; Table 2). The top 5 genes are the most frequent in all of the panel studies reported [40]. The higher apparent rates from the Lynch syndrome mismatch repair genes *PMS2, MSH6, MLH1*, and *MSH2* have been reported in a number of studies, but none show evidence of an increase in the unbiased Prospective Lynch Syndrome Database (PLSD) [100]. This may reflect bias in ascertainment towards families with additional cancers in the individual or family consistent with Lynch syndrome such as colorectal cancer. Controls from the BRIDGES study may also not be matched to the population and excluded missense variants and CNV which inflate the OR for many of the genes but especially *PMS2* and *MSH2* [101]. Although the BRIDGES study did find borderline significance for *MSH6*, none of the other mismatch repair genes were confirmed [47]. We would suggest limiting testing to a panel of *BRCA1*, *BRCA2, PALB2, ATM, CHEK2, PTEN*, and *TP53*, leaving *TP53* out if the patient is aged over 45 years and does not fulfill the Chompret criteria. *CDH1* should only be tested if the case is lobular and there is a family or personal history of gastric cancer or lobular breast cancer [43]. This will limit the number of variants of uncertain significance while identifying the vast majority of actionable genes. There could be a case for adding *BARD1, RAD51C*, and *RAD51D* for triple-negative breast cancer [47].

Interaction of Genetic and Environmental Risk Factors

Past history may not be an adequate guide for future events. In a number of families for which data are available, the age of onset of cancer seems to decline over several generations and its frequency increases. This apparent trend might be explained by selection bias, but data from Iceland again suggest that this is real and can be attributed to the environmental and lifestyle factors that account for the rising incidence of breast cancer in populations in most economically advanced countries of northern Europe and North America. At present, most evidence suggests that standard risk factors such as reproductive history, breast-feeding, use of oral contraceptives or hormone replacement therapy, diet, alcohol consumption, or any other lifestyle factor also influences the cancer risk (penetrance) of carriers of breast cancer gene mutations. For example, the penetrance of the Icelandic *BRCA2* founder mutation increased from 20 to 80% during the 20th century [102].

Contralateral Breast Cancer Risk (SNP and Known High-Risk Single Genes)

With improved breast cancer survival and high breast cancer incidence, some women are at an increased risk of developing a contralateral breast cancer. Whilst the overall risk is 0.4–0.5% per annum, estimates of likelihood are influenced by various factors including germline genetics [103].

Where a high-risk single gene disorder is present, prospective cohort studies have shown a contralateral breast cancer risk of ~2% per annum for *BRCA1* and 1–2% per annum for *BRCA2* [11]. The lower incidence for *BRCA2* likely reflects that the breast cancers occurring in *BRCA2* mutation carriers tend to be estrogen receptor positive where therapeutic endocrine therapy is indicated. Oophorectomy, particularly undertaken at a younger age (< 45 years), is associated with a significant contralateral breast cancer risk reduction in *BRCA2* carriers [10]. For the rarer, but high-risk, equivalent single gene alterations (Fig. 2), prospective contralateral breast cancer risk data is lacking, although a pragmatic *BRCA1/2* equivalent high-risk approach is offered in the clinic. Nonetheless,

for *TP53* carriers diagnosed at age < 35 years, contralateral risks appear higher than for *BRCA1* and *BRCA2* [104] and there is evidence of a relatively high rate of contralateral breast cancer for the moderate-risk genes *ATM* and *CHEK2* and a higher frequency of synchronous bilateral disease [105, 106].

Considering that collectively SNP account for a greater proportion of the familial risk of breast cancer, on a breast cancer population basis, it is those with women with a higher-risk PRS profile who are more likely to contribute to the population of contralateral breast cancers. For an SNP profile based on 313 variants, the contralateral lifetime breast cancer risk ranges from 12 to 20%, depending on the initial risk percentile [107].

Germline Genetics and Novel Therapeutic Strategies

Alongside the expansion of genetic testing capabilities has been the development of, and subsequent clinical trials involving, poly (ADP-ribose) polymerase (PARP) inhibitors, agents which render a cell unable to repair single-stranded DNA breaks. Where there is also defective homologous recombination to repair double-stranded breaks, cell lethality results. These agents are prime candidates for treatment of advanced breast cancers associated with germline or acquired mutations of *BRCA1/2* and potentially also *PALB2* given their functional roles in homologous recombination pathways. With advances in genomic technologies enabling improved turnaround times and testing being offered in mainstream clinical settings, identification of a germline *BRCA1/2* mutation in the oncology setting has important therapeutic implications, with PARP inhibition of advanced breast cancers being associated with increased progression-free survival over standard care [108, 109]. Given their use now in a maintenance setting in ovarian cancer, they well be employed after primary treatment even for earlier-stage breast cancer.

Conclusion

Breast cancer predisposition is complex, being influenced by multiple genetic and environmental factors, and our knowledge of the genetic predisposition landscape has changed markedly over the past 30 years. Thus, PV of *BRCA1* and *BRCA2* are more common than originally thought, and the associated cancer risks have been shown to be influenced by both environmental factors and lower-risk susceptibility alleles (SNP). While considerable effort has been made toward the identification of further breast cancer predisposition genes, collectively known SNP and currently unidentified genetic modifiers of risk are likely to account for the remaining heritability. With advances in genomic technologies, more widespread genetic testing is now available but needs to be concomitant with accurate variant interpretation and family follow-up. Looking to the future, the challenges that lie ahead will include the incorporation of low-risk allele detection in the clinic and accurate risk stratification so that cancer prevention and early detection strategies can be put in place, especially for contralateral risk in newly identified cases.

Conflict of Interest Statement

D.G.E. has undertaken consultancy work for Astrazeneca and Springworks.

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Author Contributions

All of the authors contributed to all aspects of this article.

References

1 Broca P. Traité des tumeurs. Paris: P. Asselin; 1866.
2 Claus EB, Risch NJ, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. Am J Epidemiol. 1990 Jun;131(6):961–72.
3 Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000 Jul;343(2):78–85.
4 Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science. 1990 Dec;250(4988):1684–9.
5 Narod SA, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, et al. Familial breast-ovarian cancer locus on chromosome 17q12-q23. Lancet. 1991 Jul;338(8759):82–3.
6 Lynch HT, Krush AJ. Carcinoma of the breast and ovary in three families. Surg Gynecol Obstet. 1971 Oct;133(4):644–8.
7 Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994 Oct;266(5182):66–71.
8 Easton DF, Ford D, Bishop DT; Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Am J Hum Genet. 1995 Jan;56(1):265–71.
9 Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al.; The Breast Cancer Linkage Consortium. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet. 1998 Mar;62(3):676–89.

10 Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, et al.; EMBRACE. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst. 2013 Jun;105(11):812–22.
11 Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al.; BRCA1 and BRCA2 Cohort Consortium. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017 Jun;317(23):2402–16.
12 Satagopan JM, Offit K, Foulkes W, Robson ME, Wacholder S, Eng CM, et al. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Cancer Epidemiol Biomarkers Prev. 2001 May;10(5):467–73.
13 Archer S, Babb de Villiers C, Scheibl F, Carver T, Hartley S, Lee A, et al. Evaluating clinician acceptability of the prototype CanRisk tool for predicting risk of breast and ovarian cancer: A multi-methods study. PLoS One. 2020 Mar;15(3):e0229999.
14 Barnes DR, Rookus MA, McGuffog L, Leslie G, Mooij TM, Dennis J, et al.; GEMO Study Collaborators; EMBRACE Collaborators; kConFab Investigators; HEBON Investigators; GENEPSO Investigators; Consortium of Investigators of Modifiers of BRCA and BRCA2. Polygenic risk scores and breast and epithelial ovarian cancer risks for carriers of BRCA1 and BRCA2 pathogenic variants. Genet Med. 2020 Oct;22(10):1653–66.
15 Mavaddat N, Barrowdale D, Andrulis IL, Domchek SM, Eccles D, Nevanlinna H, et al.; HEBON; EMBRACE; GEMO Study Collaborators; kConFab Investigators; SWE-BRCA Collaborators; Consortium of Investigators of Modifiers of BRCA1/2. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomarkers Prev. 2012 Jan;21(1):134–47.
16 Evans DG, Young K, Bulman M, Shenton A, Wallace A, Lalloo F. Probability of BRCA1/2 mutation varies with ovarian histology: results from screening 442 ovarian cancer families. Clin Genet. 2008 Apr;73(4):338–45.
17 Evans DG, Harkness EF, Plaskocinska I, Wallace AJ, Clancy T, Woodward ER, et al. Pathology update to the Manchester Scoring System based on testing in over 4000 families. J Med Genet. 2017 Oct;54(10):674–81.
18 Moran A, O'Hara C, Khan S, Shack L, Woodward E, Maher ER, et al. Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. Fam Cancer. 2012 Jun;11(2):235–42.
19 Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE; Breast Cancer Linkage Consortium. Risks of cancer in BRCA1-mutation carriers. Lancet. 1994 Mar;343(8899):692–5.
20 Thompson D, Easton DF; Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst. 2002 Sep;94(18):1358–65.
21 Sopik V, Phelan C, Cybulski C, Narod SA. BRCA1 and BRCA2 mutations and the risk for colorectal cancer. Clin Genet. 2015 May;87(5):411–8.
22 Saule C, Mouret-Fourme E, Briaux A, Becette V, Rouzier R, Houdayer C, et al. Risk of Serous Endometrial Carcinoma in Women With Pathogenic BRCA1/2 Variant After Risk-Reducing Salpingo-Oophorectomy. J Natl Cancer Inst. 2018 Feb;110(2):213–5.
23 Evans DG, Clancy T, Hill J, Tischkowitz M. Is there really an increased risk of early colorectal cancer in women with BRCA1 pathogenic mutations? Clin Genet. 2016 Mar;89(3):399.
24 Kitson SJ, Bafligil C, Ryan NA, Lalloo F, Woodward ER, Clayton RD, et al. BRCA1 and BRCA2 pathogenic variant carriers and endometrial cancer risk: A cohort study. Eur J Cancer. 2020 Sep;136:169–75.
25 Silvestri V, Leslie G, Barnes DR, Agnarsson BA, Aittomäki K, Alducci E, et al.; CIMBA Group. Characterization of the Cancer Spectrum in Men With Germline BRCA1 and BRCA2 Pathogenic Variants: Results From the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). JAMA Oncol. 2020 Aug;6(8):1218–30.
26 Page EC, Bancroft EK, Brook MN, Assel M, Hassan Al Battat M, Thomas S, et al.; IMPACT Study Collaborators. Interim Results from the IMPACT Study: Evidence for Prostate-specific Antigen Screening in BRCA2 Mutation Carriers. Eur Urol. 2019 Dec;76(6):831–42.
27 Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995 Dec;378(6559):789–92.
28 Evans DG, Shenton A, Woodward E, Lalloo F, Howell A, Maher ER. Penetrance estimates for BRCA1 and BRCA2 based on genetic testing in a Clinical Cancer Genetics service setting: risks of breast/ovarian cancer quoted should reflect the cancer burden in the family. BMC Cancer. 2008 May;8(1):155.
29 Evans DG, Susnerwala I, Dawson J, Woodward E, Maher ER, Lalloo F. Risk of breast cancer in male BRCA2 carriers. J Med Genet. 2010 Oct;47(10):710–1.
30 Lecarpentier J, Silvestri V, Kuchenbaecker KB, Barrowdale D, Dennis J, McGuffog L, et al.; EMBRACE; GEMO Study Collaborators; HEBON; KConFab Investigators. Prediction of Breast and Prostate Cancer Risks in Male BRCA1 and BRCA2 Mutation Carriers Using Polygenic Risk Scores. J Clin Oncol. 2017 Jul;35(20):2240–50.
31 Breast Cancer Linkage C; Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst. 1999 Aug;91(15):1310–6.
32 van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, et al.; Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON). Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med Genet. 2005 Sep;42(9):711–9.
33 Ginsburg OM, Kim-Sing C, Foulkes WD, Ghadirian P, Lynch HT, Sun P, et al.; Hereditary Breast Cancer Clinical Study Group. BRCA1 and BRCA2 families and the risk of skin cancer. Fam Cancer. 2010 Dec;9(4):489–93.
34 Scott RJ, Vajdic CM, Armstrong BK, Ainsworth CJ, Meldrum CJ, Aitken JF, et al. BRCA2 mutations in a population-based series of patients with ocular melanoma. Int J Cancer. 2002 Nov;102(2):188–91.
35 Malkin D. p53 and the Li-Fraumeni syndrome. Cancer Genet Cytogenet. 1993 Apr;66(2):83–92.
36 Evans DG, Moran A, Hartley R, Dawson J, Bulman B, Knox F, et al. Long-term outcomes of breast cancer in women aged 30 years or younger, based on family history, pathology and BRCA1/BRCA2/TP53 status. Br J Cancer. 2010 Mar;102(7):1091–8.
37 Frebourg T, Bajalica Lagercrantz S, Oliveira C, Magenheim R, Evans DG. European Reference Network G. Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. Eur J Hum Genet. 2020 May;28(10):1379–86.
38 Evans DG, Woodward ER. New surveillance guidelines for Li-Fraumeni and hereditary TP53 related cancer syndrome: implications for germline TP53 testing in breast cancer. Fam Cancer. 2021 Jan;20(1):1–7.
39 Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet. 1997 May;16(1):64–7.
40 Yadav S, LaDuca H, Polley EC, Hu C, Niguidula N, Shimelis H, et al. Racial and ethnic differences in multigene hereditary cancer panel test results for women with breast cancer. J Natl Cancer Inst. 2020. doi: 10.1093/jnci/djaa167.
41 van Lier MG, Westerman AM, Wagner A, Looman CW, Wilson JH, de Rooij FW, et al. High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome. Gut. 2011 Feb;60(2):141–7.
42 Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V, et al.; International Gastric Cancer Linkage Consortium. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. 2010 Jul;47(7):436–44.
43 Taylor A, Brady AF, Frayling IM, Hanson H, Tischkowitz M, Turnbull C, et al.; UK Cancer Genetics Group (UK-CGG). Consensus for genes to be included on cancer panel tests offered by UK genetics services: guidelines of the UK Cancer Genetics Group. J Med Genet. 2018 Jun;55(6):372–7.
44 Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al.; Breast Cancer Susceptibility Collaboration (UK). PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet. 2007 Feb;39(2):165–7.
45 Yang X, Leslie G, Doroszuk A, Schneider S, Allen J, Decker B, et al. Cancer Risks Associated With Germline PALB2 Pathogenic Variants: An International Study of 524 Families. J Clin Oncol. 2020 Mar;38(7):674–85.
46 Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, et al.; Breast Cancer Susceptibility Collaboration (UK). ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet. 2006 Aug;38(8):873–5.

47 Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C, et al. Breast cancer risk genes: association analysis of rare coding variants in 34 genes in 60,466 cases and 53,461 controls. N Engl J Med. Forthcoming 2021.

48 Bernstein JL, Teraoka S, Southey MC, Jenkins MA, Andrulis IL, Knight JA, et al. Population-based estimates of breast cancer risks associated with ATM gene variants c.7271T>G and c.1066-6T>G (IVS10-6T>G) from the Breast Cancer Family Registry. Hum Mutat. 2006 Nov;27(11):1122–8.

49 Goldgar DE, Healey S, Dowty JG, Da Silva L, Chen X, Spurdle AB, et al.; BCFR; kConFab. Rare variants in the ATM gene and risk of breast cancer. Breast Cancer Res. 2011 Jul; 13(4):R73.

50 Fletcher O, Johnson N, dos Santos Silva I, Orr N, Ashworth A, Nevanlinna H, et al.; kConFab Investigators; AOCS Group; GENICA Consortium; Breast Cancer Association Consortium. Missense variants in ATM in 26,101 breast cancer cases and 29,842 controls. Cancer Epidemiol Biomarkers Prev. 2010 Sep; 19(9):2143–51.

51 Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, et al.; CHEK2-Breast Cancer Consortium. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet. 2002 May;31(1):55–9.

52 Muranen TA, Greco D, Blomqvist C, Aittomäki K, Khan S, Hogervorst F, et al.; NBCS Investigators; kConFab/AOCS Investigators. Genetic modifiers of CHEK2*1100delC-associated breast cancer risk. Genet Med. 2017 May;19(5):599–603.

53 Sharif S, Moran A, Huson SM, Iddenden R, Shenton A, Howard E, et al. Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. J Med Genet. 2007 Aug;44(8):481–4.

54 Howell SJ, Hockenhull K, Salih Z, Evans DG. Increased risk of breast cancer in neurofibromatosis type 1: current insights. Breast Cancer (Dove Med Press). 2017 Aug;9:531–6.

55 Uusitalo E, Kallionpää RA, Kurki S, Rantanen M, Pitkäniemi J, Kronqvist P, et al. Breast cancer in neurofibromatosis type 1: overrepresentation of unfavourable prognostic factors. Br J Cancer. 2017 Jan;116(2):211–7.

56 Evans DG, Kallionpää RA, Clementi M, Trevisson E, Mautner VF, Howell SJ, et al. Breast cancer in neurofibromatosis 1: survival and risk of contralateral breast cancer in a five country cohort study. Genet Med. 2020 Feb; 22(2):398–406.

57 Evans DG, Howell SJ, Frayling IM, Peltonen J. Gene panel testing for breast cancer should not be used to confirm syndromic gene associations. NPJ Genom Med. 2018 Nov;3(1):32.

58 Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, et al.; Breast Cancer Susceptibility Collaboration (UK). Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. Nat Genet. 2006 Nov; 38(11):1239–41.

59 Easton DF, Lesueur F, Decker B, Michailidou K, Li J, Allen J, et al.; Australian Ovarian Cancer Study Group; kConFab Investigators; Lifepool Investigators; NBCS Investigators. No evidence that protein truncating variants in BRIP1 are associated with breast cancer risk: implications for gene panel testing. J Med Genet. 2016 May;53(5):298–309.

60 Dong H, Chandratre K, Qin Y, Zhang J, Tian X, Rong C, et al. Prevalence of BRCA1/BRCA2 pathogenic variation in Chinese Han population. J Med Genet. 2020. doi: 10.1136/jmedgenet-2020-106970.

61 Anglian Breast Cancer Study Group. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Br J Cancer. 2000 Nov; 83(10):1301–8.

62 Li J, Wen WX, Eklund M, Kvist A, Eriksson M, Christensen HN, et al. Prevalence of BRCA1 and BRCA2 pathogenic variants in a large, unselected breast cancer cohort. Int J Cancer. 2019 Mar;144(5):1195–204.

63 Evans DG, van Veen EM, Byers HJ, Wallace AJ, Ellingford JM, Beaman G, et al. A Dominantly Inherited 5′ UTR Variant Causing Methylation-Associated Silencing of BRCA1 as a Cause of Breast and Ovarian Cancer. Am J Hum Genet. 2018 Aug;103(2):213–20.

64 Byers H, Wallis Y, van Veen EM, Lalloo F, Reay K, Smith P, et al. Sensitivity of BRCA1/2 testing in high-risk breast/ovarian/male breast cancer families: little contribution of comprehensive RNA/NGS panel testing. Eur J Hum Genet. 2016 Nov;24(11):1591–7.

65 Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, Pooley KA, et al.; Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer; Breast Cancer Association Consortium. A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet. 2007 Mar;39(3):352–8.

66 Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al.; SEARCH collaborators; kConFab; AOCS Management Group. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007 Jun;447(7148): 1087–93.

67 Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet. 2007 Jul; 39(7):870–4.

68 Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet. 2007 Jul;39(7):865–9.

69 Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, Jonsson GF, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet. 2008 Jun;40(6): 703–6.

70 Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, Platte R, et al.; SEARCH; GENICA Consortium; kConFab; Australian Ovarian Cancer Study Group. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. Nat Genet. 2009 May;41(5): 585–90.

71 Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, Cox DG, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet. 2009 May; 41(5):579–84.

72 Zheng W, Long J, Gao YT, Li C, Zheng Y, Xiang YB, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet. 2009 Mar; 41(3):324–8.

73 Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, et al.; Breast Cancer Susceptibility Collaboration (UK). Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet. 2010 Jun;42(6):504–7.

74 Fletcher O, Johnson N, Orr N, Hosking FJ, Gibson LJ, Walker K, et al. Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. J Natl Cancer Inst. 2011 Mar;103(5):425–35.

75 Haiman CA, Chen GK, Vachon CM, Canzian F, Dunning A, Millikan RC, et al.; Gene Environment Interaction and Breast Cancer in Germany (GENICA) Consortium. A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. Nat Genet. 2011 Oct;43(12): 1210–4.

76 Siddiq A, Couch FJ, Chen GK, Lindström S, Eccles D, Millikan RC, et al.; Australian Breast Cancer Tissue Bank Investigators; Familial Breast Cancer Study; GENICA Consortium. A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11. Hum Mol Genet. 2012 Dec;21(24):5373–84.

77 Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet. 2013 Apr;45(4):371–84, 84e1–2.

78 French JD, Ghoussaini M, Edwards SL, Meyer KB, Michailidou K, Ahmed S, et al.; GENICA Network; kConFab Investigators. Functional variants at the 11q13 risk locus for breast cancer regulate cyclin D1 expression through long-range enhancers. Am J Hum Genet. 2013 Apr;92(4):489–503.

79 Garcia-Closas M, Couch FJ, Lindstrom S, Michailidou K, Schmidt MK, Brook MN, et al. Genome-wide association studies identify four ER negative-specific breast cancer risk loci. Nat Genet. 2013 Apr;45(4):392–8, 398e1–2.

80 Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013 Apr;45(4):353–61, 361e1–2.

81 Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, et al.; BOCS; kConFab Investigators; AOCS Group; NBCS; GENICA Network. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet. 2015 Apr;47(4):373–80.
82 Michailidou K, Lindström S, Dennis J, Beesley J, Hui S, Kar S, et al.; NBCS Collaborators; ABCTB Investigators; ConFab/AOCS Investigators. Association analysis identifies 65 new breast cancer risk loci. Nature. 2017 Nov; 551(7678):92–4.
83 Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, et al.; ABCTB Investigators; kConFab/AOCS Investigators; NBCS Collaborators. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. Am J Hum Genet. 2019 Jan;104(1): 21–34.
84 Antoniou AC, Wang X, Fredericksen ZS, McGuffog L, Tarrell R, Sinilnikova OM, et al.; EMBRACE; GEMO Study Collaborators; HEBON; kConFab; SWE-BRCA; MOD SQUAD; GENICA. A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet. 2010 Oct;42(10):885–92.
85 Couch FJ, Kuchenbaecker KB, Michailidou K, Mendoza-Fandino GA, Nord S, Lilyquist J, et al. Identification of four novel susceptibility loci for oestrogen receptor negative breast cancer. Nat Commun. 2016 Apr;7(1):11375.
86 Dunning AM, Michailidou K, Kuchenbaecker KB, Thompson D, French JD, Beesley J, et al.; EMBRACE; GEMO Study Collaborators; HEBON; kConFab Investigators. Breast cancer risk variants at 6q25 display different phenotype associations and regulate ESR1, RMND1 and CCDC170. Nat Genet. 2016 Apr;48(4):374–86.
87 Milne RL, Kuchenbaecker KB, Michailidou K, Beesley J, Kar S, Lindström S, et al.; ABCTB Investigators; EMBRACE; GEMO Study Collaborators; HEBON; kConFab/AOCS Investigators; NBSC Collaborators. Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. Nat Genet. 2017 Dec;49(12):1767–78.
88 Evans DG, Warwick J, Astley SM, Stavrinos P, Sahin S, Ingham S, et al. Assessing individual breast cancer risk within the U.K. National Health Service Breast Screening Program: a new paradigm for cancer prevention. Cancer Prev Res (Phila). 2012 Jul;5(7):943–51.
89 Brentnall AR, Evans DG, Cuzick J. Distribution of breast cancer risk from SNPs and classical risk factors in women of routine screening age in the UK. Br J Cancer. 2014 Feb; 110(3):827–8.
90 Dite GS, MacInnis RJ, Bickerstaffe A, Dowty JG, Allman R, Apicella C, et al. Breast Cancer Risk Prediction Using Clinical Models and 77 Independent Risk-Associated SNPs for Women Aged Under 50 Years: Australian Breast Cancer Family Registry. Cancer Epidemiol Biomarkers Prev. 2016 Feb;25(2):359–65.
91 Cuzick J, Brentnall AR, Segal C, Byers H, Reuter C, Detre S, et al. Impact of a Panel of 88 Single Nucleotide Polymorphisms on the Risk of Breast Cancer in High-Risk Women: Results From Two Randomized Tamoxifen Prevention Trials. J Clin Oncol. 2017 Mar; 35(7):743–50.
92 Evans DG, Brentnall A, Byers H, Harkness E, Stavrinos P, Howell A, et al.; FH-risk study Group. The impact of a panel of 18 SNPs on breast cancer risk in women attending a UK familial screening clinic: a case-control study. J Med Genet. 2017 Feb;54(2):111–3.
93 van Veen EM, Brentnall AR, Byers H, Harkness EF, Astley SM, Sampson S, et al. Use of Single-Nucleotide Polymorphisms and Mammographic Density Plus Classic Risk Factors for Breast Cancer Risk Prediction. JAMA Oncol. 2018 Apr;4(4):476–82.
94 Brentnall AR, van Veen EM, Harkness EF, Rafiq S, Byers H, Astley SM, et al. A case-control evaluation of 143 single nucleotide polymorphisms for breast cancer risk stratification with classical factors and mammographic density. Int J Cancer. 2020 Apr;146(8): 2122–9.
95 Ho WK, Tan MM, Mavaddat N, Tai MC, Mariapun S, Li J, et al. European polygenic risk score for prediction of breast cancer shows similar performance in Asian women. Nat Commun. 2020 Jul;11(1):3833.
96 Garrett A, Callaway A, Durkie M, Cubuk C, Alikian M, Burghel GJ, et al.; CanVIG-UK. Cancer Variant Interpretation Group UK (CanVIG-UK): an exemplar national subspecialty multidisciplinary network. J Med Genet. 2020 Dec;57(12):829–34.
97 Flaum N, Morgan RD, Burghel GJ, Bulman M, Clamp AR, Hasan J, et al. Mainstreaming germline BRCA1/2 testing in non-mucinous epithelial ovarian cancer in the North West of England. Eur J Hum Genet. 2020 Nov;28(11): 1541–7.
98 Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, et al. High prevalence of the 999del5 mutation in icelandic breast and ovarian cancer patients. Cancer Res. 1996 Aug;56(16): 3663–5.
99 Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, Nathanson KL, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med. 2015 Jun;372(23):2243–57.
100 Dominguez-Valentin M, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genet Med. 2020 Jan;22(1):15–25.
101 Smith MJ, Urquhart JE, Harkness EF, Miles EK, Bowers NL, Byers HJ, et al. The Contribution of Whole Gene Deletions and Large Rearrangements to the Mutation Spectrum in Inherited Tumor Predisposing Syndromes. Hum Mutat. 2016 Mar;37(3):250–6.
102 Tryggvadottir L, Sigvaldason H, Olafsdottir GH, Jonasson JG, Jonsson T, Tulinius H, et al. Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920-2000. J Natl Cancer Inst. 2006 Jan;98(2):116–22.
103 Kramer I, Schaapveld M, Oldenburg HS, Sonke GS, McCool D, van Leeuwen FE, et al. The Influence of Adjuvant Systemic Regimens on Contralateral Breast Cancer Risk and Receptor Subtype. J Natl Cancer Inst. 2019 Jul;111(7):709–18.
104 Hyder Z, Harkness EF, Woodward ER, Bowers NL, Pereira M, Wallace AJ, et al. Risk of Contralateral Breast Cancer in Women with and without Pathogenic Variants in BRCA1, BRCA2, and TP53 Genes in Women with Very Early-Onset (<36 Years) Breast Cancer. Cancers (Basel). 2020 Feb;12(2):E378.
105 de Bock GH, Schutte M, Krol-Warmerdam EM, Seynaeve C, Blom J, Brekelmans CT, et al. Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant. J Med Genet. 2004 Oct;41(10):731–5.
106 Bernstein JL, Haile RW, Stovall M, Boice JD Jr, Shore RE, Langholz B, et al.; WECARE Study Collaborative Group. Radiation exposure, the ATM Gene, and contralateral breast cancer in the women's environmental cancer and radiation epidemiology study. J Natl Cancer Inst. 2010 Apr;102(7):475–83.
107 Kramer I, Hooning MJ, Mavaddat N, Hauptmann M, Keeman R, Steyerberg EW, et al.; NBCS Collaborators; ABCTB Investigators; kConFab Investigators. Breast Cancer Polygenic Risk Score and Contralateral Breast Cancer Risk. Am J Hum Genet. 2020 Nov; 107(5):837–48.
108 Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med. 2017 Aug; 377(6):523–33.
109 Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. N Engl J Med. 2018 Aug;379(8):753–63.