

Genetic Markers in Breast Cancer – How Far Have We Come from BRCA1?

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ABSTRACT

Breast cancer is the most common malignancy that develops in women worldwide, its incidence continues to rise and it is responsible for the highest death rates. Breast cancer can be classified as sporadic or familial – the strongest risk factor today is a family history. Germline mutations in high-penetrance breast cancer susceptibility genes *BRCA1* and *BRCA2* have been strongly implicated in the genetic predisposition of approximately 20% of familial breast cancers. Although *BRCA1* and *BRCA2* do not account for all familial breast cancers, there are currently no other genes that have been identified which segregate with familial breast cancer as strongly. Despite large-scale attempts to identify genetic risk factors associated with breast cancer, the variants identified through genome-wide association studies (GWAS), only confer a modest increase in risk of breast cancer and at present lack clinical utility. This review will discuss the known genetic risk factors for developing breast cancer and how far the field has progressed since the identification of *BRCA1*.

INTRODUCTION

Breast cancer is the most common malignancy that develops in women worldwide, its incidence continues to rise and it is responsible for the highest death rates [1]. Breast cancer is a disease with complex aetiology with multiple predisposing factors. There is a substantial body of experimental, clinical and epidemiological evidence indicating that hormones play a major role in the development of breast cancer and this has been extensively reviewed in the literature [2-6]. Firstly, breast cancer in men is rare, suggesting an influence of the female sex steroids [7]. Secondly, in studies conducted as early as 1896, it was reported that removal of the ovaries caused the regression of breast cancer, and subsequent studies, many years later, reported that the estrogen antagonist, Tamoxifen, had proven efficacy in the treatment of advanced breast cancer and in the reduction of breast cancer risk when used in an adjuvant setting [3, 8, 9]. Thirdly, it has been repeatedly demonstrated that estrogen and progesterone treatment in animal models, including rodents and monkeys, can promote mammary carcinogenesis and this is critically dependent on functional estrogen and progesterone receptors (ER and PR) [3-5]. Finally, there have been numerous epidemiological studies which have reported increases in breast cancer risk in women with increased exposure to endogenous and exogenous hormones [10, 11]. Endogenous and exogenous hormones can promote

tumour formation by driving cell proliferation, subsequently increasing the number of cell divisions as well as the opportunity for random genetic errors [2]. Alternatively, estrogen metabolites may directly generate DNA damage, thus leading to genomic instability [12, 13]. However, there is also strong evidence for a genetic component to breast cancer, the strongest risk factor today is a family history. The genetic basis for the inherited predisposition to breast cancer has been ardently investigated in the past two decades and has resulted in the discovery of several high- to low-penetrance breast cancer susceptibility genes through genome-wide linkage studies and mutational screening of candidate genes in large breast cancer case series. This review will discuss the known genetic risk factors for developing breast cancer with a particular focus on *BRCA1*.

HIGHLY PENETRANT BREAST CANCER SUSCEPTIBILITY GENES

BRCA1/2

Hereditary breast cancer accounts for a small but significant proportion (12.9%) of all breast cancers [14]. Analysis of pedigrees from high-risk breast/ovarian cancer families led to the identification and cloning of the first breast cancer susceptibility gene (*BRCA1*) [15, 16] spanning approximately 100kb of genomic DNA and consisting of 24

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exons (although exons 1 and 4 are non-coding), that encode a large multi-domain protein of 1863aa [16]. The role of this protein in genetic susceptibility to breast and ovarian cancer was confirmed when germline mutations in *BRCA1* were identified in individuals with a family history of breast and/or ovarian cancer [16-18]. In addition to an elevated risk of breast and ovarian cancer, *BRCA1* mutation carriers were also found to have an increased risk of colon, pancreatic, endometrial, cervical and prostate cancer [19-23]. However, the risk of cancer at these sites is relatively small when compared to the risk of cancer in the breast or ovary. Direct evidence that *BRCA1* acted as a tumour suppressor in human breast and ovarian cancer cells came from *in vitro* studies where its over-expression retarded cell growth and its inhibition accelerated cell growth, while mutated forms of the protein had no effect [24, 25]. A second breast cancer susceptibility gene, *BRCA2* was subsequently identified in 1994 [26].

The histopathology of breast cancers diagnosed in *BRCA1* mutation carriers differ substantially from those that occur in sporadic disease. Breast cancers that develop in *BRCA1* mutation carriers are diagnosed at younger age, are of higher grade, often exhibit higher mitotic counts and have a greater proportion of tumour with continuous pushing margin [27-36]. Breast tumours in *BRCA1* mutation carriers are more likely to be negative for ER, PR and AR when compared to sporadic breast cancers [34, 37-44]. They contain less tubule formation, more nuclear polymorphism and more lymphocytic infiltrate compared with their sporadic counterparts [29-31]. In addition, *BRCA1* mutation carriers are more likely to develop contralateral breast cancer [27, 39]. Premalignant lesions associated with increased malignancy, such as ductal carcinoma in situ (DCIS), atypical ductal hyperplasia (ADH), and atypical lobular carcinoma (ALH), may be more prevalent in the normal breast tissue from *BRCA1* mutation carriers compared to the breast of patients without a known genetic predisposition to the disease [45-48]. Thus, women with an inherited predisposition to breast cancer due to a *BRCA1* mutation often present clinically with breast tumours containing features associated with a highly proliferative phenotype and with a worse prognosis.

To date, more than 1600 distinct pathogenic mutations, polymorphisms and variants in the *BRCA1* gene have been identified. In addition, over 1800 distinct pathogenic mutations have been identified in *BRCA2* [49]. The mutations are scattered throughout the entire coding region of the genes and show no clustering or "hot spots". The frequency of these mutations varies widely among different populations and is influenced by founder effects. For example, in the Ashkenazi Jewish community four mutations are thought to account for the majority of inherited breast and/or ovarian cancer: 185delAG, 188del11 and 5382insC in the *BRCA1* gene and 6174delT in the *BRCA2* gene [50]. In original analyses by the Breast Cancer Linkage Consortium (BCLC), *BRCA1* mutations were shown to occur in > 90% of families that contained at least four breast and/or ovarian

cancers; and were estimated to occur in 45% of sporadic cases [51, 52]. However, since the cloning of this gene [16], the prevalence estimates for *BRCA1* have been much less than those estimated by initial linkage studies.

In hereditary breast cancer, mutations in *BRCA1* have been shown to occur in the large proportion of cases (~30-80%) in families with both breast and ovarian cancer, whereas *BRCA2* mutations account for a smaller proportion (~15-20%) [52-58]. In families containing breast cancer only, *BRCA1* mutations occur in around 7% of cases where there is more than one first degree relative and in 28% of cases where there are four or more affected individuals within a family [53, 59]. *BRCA1* mutations are more prevalent in ovarian cancer only families (compared to breast cancer only families) and are detected in 31-38% of cases [60-62]. The prevalence of *BRCA1* mutations in population-based studies is considerably lower than those estimated for large multiple-case families. Perhaps the most reliable estimate to date comes from a study conducted by Antoniou and colleagues, where population-based cohorts from 22 previous studies in 13 different countries were analysed. From 8139 index cases presenting with breast or ovarian cancer, 3.5% were found to carry a mutation in *BRCA1* and 2.7% carried a mutation in *BRCA2* [63]. Moreover, breast cancer incidences were shown to increase with age, up to age 45-49 years in *BRCA1*-mutation carriers, but reached a plateau thereafter, whereas breast cancer incidence *BRCA2*-mutation carriers increased gradually with age [23, 60, 64].

Although *BRCA1* and 2 mutations are found in a high proportion of familial breast/ovarian cancer patients, defining the penetrance (the risk that a given mutation will lead to cancer) of *BRCA1* mutations remains a challenging task. Early studies of large multiple-case families suggested that the lifetime risk of disease may be as high as 85% in *BRCA1* or *BRCA2* mutation carriers [19, 51-53]. However, more recent analyses have shown significantly lower risks than those initially estimated, 65% for the risk of breast cancer development and 39% for the risk of ovarian cancer development up to 70 years of age for *BRCA1* mutation carriers [23, 60, 64]. The risk estimates for developing breast or ovarian cancer for *BRCA2* mutation carriers are lower than that of *BRCA1* mutation carriers at 45% (breast) and 11% (ovarian) respectively [23, 60, 64]. Nevertheless, regardless of the precise penetrance estimate, *BRCA1* and 2 are highly penetrant (albeit not completely) breast cancer susceptibility genes whose mutation significantly increases the risk of developing breast cancer.

DOES BRCA1 MUTATION POSITION ALTER DISEASE PHENOTYPE?

A critical question in mutation screening of *BRCA1* is whether the position of the mutation correlates with the cancer phenotype (i.e. breast or ovarian cancer). Knowing the biological consequence of a particular mutation could provide valuable information to patients seeking counselling on which type of preventative and management

measures to undertake, if a mutation is found. The first formal evidence linking risk to mutation position came from Gayther et al. (1995) who found that for 22 different mutations in 32 families, the ratio of breast to ovarian cancer was significantly lower after a “change point”. This change point was codon 1435 in exon 13, where a *BRCA1* mutation 5’ of this codon conferred a significantly higher risk of developing ovarian cancer [65]. This has been confirmed by a study conducted in the UK [66], however, other studies have failed to confirm a relationship between mutation position and cancer phenotype [67, 68].

In a population-based study of women with *BRCA1*-related ovarian cancer, the risk of breast cancer in first degree relatives was found to increase proportionally as the mutation position approached the carboxyl terminus of the gene [69]. In contrast to previous reports, a study conducted by the Breast Cancer Linage Consortium of 356 *BRCA1*-related families found that mutations in the central portion of the gene (nucleotides 2401-4190) had a significantly higher ovarian: breast cancer ratio when compared to mutations between nucleotides 1-2400 or nucleotides 4191-end [70]. This was supported by an Australian study which, although subjects with ovarian cancer were not included, found that mutations within the central portion of the gene (2356-4592) were associated with a decreased risk of breast cancer, compared to mutations at the N- and C-terminus [64]. Furthermore, the same genotype-phenotype relationship was also observed in a meta-analysis of 22 previous studies, although statistical significance was not achieved [63].

Although it is possible that the position of the mutation within *BRCA1* will affect the prevalence of breast or ovarian cancer, there is no solid evidence as yet, to suggest that this is the case. The available research is limited and has produced conflicting results as to which mutated regions present greater risk for breast or ovarian cancer.

OTHER HIGH PENETRANCE BREAST CANCER SUSCEPTIBILITY GENES

High penetrance breast cancer susceptibility genes, other than *BRCA1/2*, have been identified as a result of their association with other inherited cancer syndromes and include *TP53*, *PTEN* and *STK11*.

The p53 tumour suppressor protein is essential for the response to DNA damage and is classed as a “guardian of the genome” [71, 72]. The involvement of the p53 protein, encoded by *TP53*, in multiple cellular pathways involved in tumour suppression has long been established, and somatic mutations in the gene are frequently observed in many types of solid tumours [73]. Germline mutations in *TP53* cause Li-Fraumeni syndrome, an inherited disorder that greatly increases susceptibility to breast and other cancers. Although if present they are highly penetrant, conferring up to a 10 fold increase in the risk of developing breast cancer, these mutations are very rare and account for a much lower familial risk than *BRCA1* and *BRCA2* [74].

Moreover, they are uncommon in non-Li-Fraumeni breast cancer families [74, 75].

Germline mutations in *PTEN* and *STK11* are known to lead to Cowden and Peutz-Jeghers syndrome respectively [76, 77]. Individuals with Cowden syndrome have an estimated 30-50% risk of developing breast cancer by the age of 70 years [76], while the risk of developing breast cancer is approximately 45% in individuals with Peutz-Jeghers syndrome [78, 79]. However, *PTEN* and *STK11* mutations are rare in high risk breast/ovarian cancer individuals that do not carry *BRCA1/2* mutations [80, 81]. Interestingly, gross aberrations in *PTEN* were found in *BRCA1* heterozygous mutation carriers and loss of *PTEN* expression was associated with basal-like sporadic breast cancer cases, indicating that its loss may be tightly linked with deficiencies in the double-strand break DNA repair pathway [82].

Together, mutations in *BRCA1/2*, *TP53*, *PTEN* and *STK11*, although highly penetrant, only account for around 20-25% of the familial risk of breast cancer. Moreover, mutations in *TP53*, *PTEN* and *STK11* are rare in patients who do not have the cancer syndromes associated with these genes and they are unlikely to contribute to a substantial fraction of breast cancer susceptibility in high-risk breast cancer families who do not harbour inactivating mutations in the *BRCA1/2* tumour suppressor genes.

VARIANTS IN BREAST CANCER SUSCEPTIBILITY GENES WITH INTERMEDIATE PENETRANCE

Four intermediate-penetrance breast cancer susceptibility genes, *CHEK2*, *ATM*, *PALB2*, and *BRIP1*, were identified through direct re-sequencing of candidate genes in familial association studies. Disease-causing mutations in these genes are predominantly ones that cause premature protein truncation, but are rare and confer a relative risk of breast cancer of 2- to 4-fold. All four genes are involved in the *BRCA1* DNA repair pathway.

ATM AND CHEK2

BRCA1 is a target for phosphorylation by several checkpoint kinases in response to DNA damage. The kinases that participate in this signalling cascade upstream of *BRCA1* include *ATM* and the checkpoint protein *CHEK2*. *ATM* is a protein kinase that is structurally related to the phosphoinositide-3 kinase (PI3K)-related kinase family and is a central component of the DNA damage response [83, 84]. *CHEK2* is activated by *ATM* and the phosphorylation of *BRCA1* by *CHEK2* has been shown to govern which pathway DNA damage will be repaired by: phosphorylation of *BRCA1* at Ser-988 promotes the repair of damaged DNA by homologous recombination (error-free), while suppressing repair by non-homologous recombination (error prone) [85]. It was demonstrated by Renwick and colleagues that monoallelic *ATM* mutations were present

in 2.04% of familial breast cancer cases and were associated with a 2.37 fold increased breast cancer risk [86]. The *CHEK2*1100delC* was found to have a frequency of 1.1% in healthy individuals and 5.1% in individuals with breast cancer from 718 *BRCA1* or *BRCA2* mutation negative families [87]. In the largest study to date, involving over 26,000 cases and 27,000 controls, heterozygosity for the *CHEK2*1100delC* was associated with a 3-5 fold increase in the risk of developing breast cancer. Furthermore, in familial breast cancer cases the *CHEK2*1100delC* was associated with a 37% lifetime risk of breast cancer, which was almost as high as the lifetime risks conferred by mutations in *BRCA1* or 2 in this study [88].

BRIP1 AND PALB2

BRIP1 and *PALB2* encode for proteins that co-localize with *BRCA1* and *BRCA2* to mediate the repair of DNA double-strand breaks [89-92]. Truncating mutations arising in these genes are rare, exhibited in approximately 0.1% of the United Kingdom breast cancer cohort, and confer an approximately two-fold increase in the risk of breast cancer compared to population risks [89, 90]. Founder mutations have been observed in French-Canadian, Finnish and Polish populations, allowing for the identification of a gene-disease association [93-95]. However, mutations in *BRIP1* and *PALB2* show incomplete segregation with disease in relatives of probands who have breast cancer [89, 96], making it difficult to use this information in predictive screening to estimate individual breast cancer risk. There is no evidence to date that monoallelic mutations in *BRIP1* and *PALB2* confer a phenotype beyond predisposition to breast cancer, although biallelic mutations in these genes are implicated in Fanconi anemia [97].

Inactivating mutations in the intermediate penetrance genes *ATM*, *CHEK2*, *BRIP1* and *PALB2* are rare, with fewer than 1% of the population being heterozygote for these variants. Collectively, mutations in these genes account for approximately 2.3% of the familial breast cancer risk [89].

THE IDENTIFICATION OF NEW LOW RISK VARIANTS

GENETIC MODIFIERS OF RISK IN THE GENERAL POPULATION

Since the identification of *BRCA1* twenty years ago [15, 16], numerous studies have used candidate gene approaches to identify other breast cancer susceptibility genes (*BRCAx*) whose mutations may lead to an increased risk of breast cancer, with limited success - *BRCAx* still remains elusive. Mutations in the high and moderate penetrance breast cancer susceptibility genes described above account for approximately 25% of breast cancers; are rarely found in sporadic cases and thus, much of the genetic component of breast cancer risk remains to be characterised. The majority of breast cancers do not arise through typical patterns of familial inheritance, they occur sporadically. Sporadic breast cancers differ markedly from familial breast cancers; with respect to their age of onset (post-menopause versus pre-menopause), their phenotype (receptor positive versus receptor negative in *BRCA1* mutation carriers) and their clinical behaviour (familial breast cancers tend to be more aggressive). Thus, candidate gene approaches in large familial pedigree studies are likely to miss common genetic variation that confers a risk of breast cancer in the majority of the population. Genome-wide association studies (GWAS) in very large study populations of breast cancer cases and unaffected controls have gained momentum in recent years, enabling an unbiased investigation of genetic variation across the whole genome, as opposed to examination of selected variants in a few key genes, in an effort to discover new breast cancer susceptibility loci. To date, 13 breast cancer susceptibility loci have been discovered by these approaches (Table 1). None of the identified variants are in the coding regions of genes, although some of the regions contain or are nearby known genes (e.g. *FGFR2*, *MAP3K1*, *RAD51L*) and five of the variants are located in non-genic regions (1p11.2, 2q35, 3p24, 5p12, 8q24) [98-104]. The

Table 1. Classes and key features of known breast cancer susceptibility genes [98-104]

Class of Breast Cancer Susceptibility Genes	Key Features			
	Genes/Alleles	Frequency	Risk of breast cancer	Risk variants
High-penetrance breast cancer susceptibility genes	<i>BRCA1</i> , <i>BRCA2</i> , <i>TP53</i>	Rare (population carrier frequency $\leq 0.1\%$)	10 to 20 fold relative risk	Multiple, different mutations that predominantly cause protein truncation
Intermediate-penetrance breast cancer susceptibility genes	<i>ATM</i> , <i>CHEK2</i> , <i>BRIP1</i> , <i>PALB2</i>	Rare (population carrier frequency $\leq 0.6\%$)	2 to 4 fold relative risk [150-153]	Multiple, different mutations that predominantly cause protein truncation
Low-penetrance breast cancer susceptibility alleles	rs2981582 (<i>FGFR2</i> , 10q), rs3803662 (<i>TOX3</i> , 16q), rs889312 (<i>MAP3K1</i> , 5q), rs3817198 (<i>LSP1</i> , 11p), rs1045485 (<i>CASP8</i> , 2q), rs13281615 (8q), rs13387042 (2q), rs10941679 (5p), rs2046210 (6q), rs4973768 (<i>SLC4A7</i> , 3p), rs6504950 (<i>COX11</i> , 17q), rs8009944 (<i>RAD51L1</i> , 14q), rs11249433 (1p)	Common (population frequency 5-50%)	up to ~1.25 fold (heterozygous) or 1.65 fold (homozygous) relative risk	Single nucleotide polymorphisms (SNPs) that are causal or in linkage disequilibrium with the causal variant

individual variants confer only a modest increase in the risk of breast cancer, of up to approximately 1.65 fold.

Importantly, candidate gene approaches and the recent GWA studies have found no evidence that common variants in high risk breast cancer susceptibility genes such as *BRCA1/2*, *TP53*, *ATM* and *CHEK2* are associated with increased breast cancer risk [105, 106]. In fact, few of the genes in the regions identified by GWAS had previously been reported to be associated with breast cancer. The strongest association with breast cancer risk was found for a SNP located in intron 2 of *FGFR2* (rs2981582), where women carrying two copies of the high risk allele were observed to have a 1.63 fold increased risk when compared to women who carried two copies of the low risk allele [98]. The FGF signalling pathway has previously been shown to induce mammary tumours in mouse models [107] and in particular, amplification of *FGFR2* has been shown to occur in 5-10% of human breast cancers [108, 109]. Recent studies by Meyer et al., have demonstrated that homozygotes for the high risk *FGFR2* allele have increased expression of *FGFR2* and functional studies have indicated that this is due to altered binding affinity of *FGFR2* for the transcription factors Oct-1 and C/EBPb [110]. This was the first study to determine a biological consequence caused by variants identified through GWA studies and clearly demonstrates a mechanism that may lead to increased breast cancer risk in individuals who are homozygous for the high risk *FGFR2* allele. However, a functional consequence for many of the variants still remains at large and the biological mechanisms underlying the observed increase in breast cancer risk are unknown.

It is apparent that each individual variant identified through GWA studies only accounts for a modest increase in the risk of developing breast cancer, suggesting that most of the unexplained breast cancer risk could be accounted for by a polygenic model, where risk alleles act multiplicatively [111]. A recent study by Reeves et al. (2010) comparing 13 risk alleles (Table 1 and *ATM*: rs1800054) in 10 306 women with breast cancer and 10 393 women without breast cancer; has investigated the combined effect 7 SNPs that were most strongly and significantly associated with breast cancer risk individually [112]. Odds ratios (ORs) were shown to increase from 0.75 to 1.45 for cases in the bottom fifth quintile compared to cases in the top fifth quintile, however, ORs in the top fifth quintile did not differ greatly from the OR of the individual *FGFR2* allele (1.20), which showed the strongest association with breast cancer risk. The cumulative risk of developing breast cancer was 8.8% for cases in the top fifth quintile compared to 4.4% for cases in the bottom fifth quintile. Thus, although a polygenic risk model for the variants has been demonstrated, the cumulative lifetime risk (8.8%) of developing breast cancer in women in the top fifth quintile was shown to be similar to that of women with one affected first-degree relative (9.1%) and far less than that of women with two affected first-degree relatives (15.4%) [112]. In addition, it has been demonstrated that 12 of the identified

variants show no interactions with other risk factors for breast cancer including HRT, age at menopause, age at menarche, BMI and parity [113]; and variants identified from GWA studies conducted by Hunter et al., [104] and Thomas et al. [100] were found to have no association with breast cancer survival [114]. Thus at this stage, polygenic risk scores are not a useful tool to inform women of their risk of developing breast cancer and appear to have limited utility in breast cancer prognostication.

GENETIC MODIFIERS OF RISK IN BRCA MUTATION CARRIERS

Given the substantial heterogeneity in penetrance estimates for *BRCA1* mutation carriers between studies of different populations, in addition to the variation in penetrance estimates observed between individuals, it is reasonable to postulate the existence of other factors which may modulate the risk of cancer in *BRCA1* mutation carriers. In this way, “modifiers” may act in conjunction with the mutated *BRCA1* allele to bring about an earlier diagnosis in an individual, thus affecting the penetrance of this gene.

Candidate gene approaches have identified several genetic variants that may alter the risk of breast cancer in *BRCA1* mutation carriers. There are several reports that allelic variants of the *Androgen Receptor (AR)* and the *Amplified In Breast Cancer 1 (AIB1)* gene, can modify the risk of breast cancer in *BRCA1* mutation carriers [115-117]. However, other studies have found no excess breast cancer risk in *BRCA1* mutation carriers according to their *AR* allele or *AIB1* polymorphism status [118-122]. *TP53* may also play a role in *BRCA1*-related tumorigenesis. *TP53* has been found to be mutated in 53-77% of *BRCA1*-related breast cancers, but only in 30-35% of sporadic breast tumours [123-127]. Likewise, *TP53* mutations are found in 50-83% of ovarian cancers linked to *BRCA1* compared to 30-49% of sporadic ovarian tumours [128-131]. Thus, the high rate of *TP53* mutations found in cancers of *BRCA1* mutation carriers indicates that inactivation of p53 function may act as a modifier of cancer risk in these women. However, subsequent studies have found no association between polymorphisms in *TP53 (Arg72Pro)* or *MDM (309G > T)* (either singly or in combination) and breast cancer risk in *BRCA1* or *BRCA2* mutation carriers, suggesting that the inactivation of *TP53* and its signalling pathway may occur as a result of tumour progression rather than being a risk modifier [132]. The *RAD51 135G > C* polymorphism has been shown to increase breast cancer risk in *BRCA2* mutation carriers by approximately 3 fold, but does not increase breast cancer risk in *BRCA1* mutation carriers or the general population [133, 134]. Instead, the *RAD51 135G > C* polymorphism may protect against the risk of breast cancer in *BRCA1* mutation carriers [135]. There is also evidence that the 677C > T polymorphism in *Methylenetetrahydrofolate reductase (MTHFR)* and the C to T transition in the 3' untranslated region of the *Prohibitin (PHB)* may increase breast cancer risk in *BRCA1*

mutation carriers [136, 137]. Allele variants of the *Insulin-like Growth Factor-1 (IGF1)* and the *Progesterone Receptor (PR)* are known to alter hormonal sensitivity and have been shown to modify the risk of breast and ovarian cancer respectively, in *BRCA1* mutation carriers [138, 139]. Polymorphisms in the *Harvey Rat Sarcoma 1 (HRAS1)* proto-oncogene as well as common variants of the *BRCA1* wild-type allele itself have been shown to increase the risk of ovarian cancers in *BRCA1* mutation carriers [140, 141]. Other groups have reported low expression of the pro-apoptotic protein *B-cell Lymphoma Protein 2 (Bcl-2)* and amplification of the *Myelocytomatosis (MYC)* oncogene, in *BRCA1*-associated breast tumours compared to sporadic cases [142, 143], but whether these genes act as risk modifiers in BRCA-related tumorigenesis is unknown.

Numerous other candidate genes have been investigated as modifiers of *BRCA1/2* penetrance, however, they have been met with limited success. Given the lack of targets found to positively correlate with breast cancer risk by candidate gene approaches in *BRCA1/2* mutation carriers, GWAS studies are now being applied to *BRCA1/2* mutation carriers. Initial studies have focussed on determining whether variants that increase breast cancer risk in population based-studies confer a similar risk of breast cancer development in *BRCA1/2* mutation carriers. To this end, five of the variants identified by GWAS (Table 1) have been genotyped in women who are *BRCA1* and 2 mutation carriers. While variants in *FGFR2*, *TNCR9*, *LSP1*, *MAP3K1* and the 2q35 region were associated with breast cancer risk in *BRCA2* mutation carriers, only the *TNCR9* variant and the 2q35 region was associated with breast cancer risk in *BRCA1* mutation carriers [144, 145]. More recently, GWA studies have been carried out in *BRCA1/2* mutation carriers to identify new modifiers of disease risk in these individuals. Interestingly, *FGFR2* was the only locus to reach GWAS statistical significance and showed a similar magnitude of risk (approximately 1.3 fold increased risk) as reported for breast cancer risk in population-based studies [146]. Thus, variants that modify breast cancer risk in the general population also appear to modify breast cancer risk in *BRCA2* mutation carriers. However, this does not appear to be the case in *BRCA1* mutation carriers where one locus (19p13) containing 5 variants has been reported to be associated with breast cancer risk in these individuals and a similar association was also reported for ER-negative and triple negative cases [147]. These results seem to reflect the distinct biology of *BRCA1*-related breast cancers, which are predominantly hormone receptor negative, compared to *BRCA2*-related and sporadic breast cancers, and further highlights the genetically divergent pathways that lead to tumorigenesis in these women.

SUMMARY AND CONCLUSIONS

Following the identification of *BRCA1* and *BRCA2* as high penetrance breast cancer susceptibility genes, significant

effort has been aimed at identifying further susceptibility genes that may account for the missing heritability to breast cancer. The advances made have been small and the elusive *BRCAx* has still not been found. This has led to the conclusion that there are in fact no other high penetrance breast cancer susceptibility genes and that the remainder of the genetic risk to breast cancer can be accounted for by a polygenic model, where many variants of low penetrance act multiplicatively to increase the relative risk of developing breast cancer. In the last 3 years, with considerable advances in technology, a multitude of data has been generated by GWAS leading to the identification of 13 new low penetrance variants that individually account for a modest increase in breast cancer risk. However, the results have been somewhat disappointing, when combined these variants still only lead to a small increase in breast cancer risk and the information generated thus far, will not be informative in a clinical setting in determining an individuals' risk of developing breast cancer and therefore, will not be useful in preventative strategies.

How far have we come from *BRCA1* in predicting breast cancer risk? To date, most of the GWAS on breast cancer have been carried out in populations unselected for ER status, grade, age, ethnicity, etc. But breast cancer is an extremely heterogeneous disease where gene-environment interactions, especially those influencing estrogen exposure, play a major role in determining disease onset and expression and this has been repeatedly demonstrated. In this regard, it has been shown that the relative risks for each of the 13 low penetrance variants are significantly influenced by ER status (*FGFR2*, *TNCR9*), grade (*FGFR2*, *TNCR9*), bilateral disease (2q35) and lobular versus ductal carcinoma (2q35) [112]. Genetically, sporadic breast cancer can be subdivided into 5 distinct sub-groups and hormone receptor status plays a major role in discriminating these sub-groups [148]. *BRCA1*-related tumours consistently group with sporadic tumours that are negative for ER, PR and HER2, "triple negative" [148]. The most recent GWAS on *BRCA1* carriers highlights the genetic heterogeneity of breast cancer, where only one locus was shown to influence breast cancer risk and this locus had not previously been associated with sporadic disease. This locus was associated with the risk of developing ER-negative breast cancer and triple-negative breast cancer, but not ER-positive breast cancer [147]. Other studies have also shown significantly stronger associations of many of the 13 identified variants with ER-positive disease compared to ER-negative disease and this is especially true of the polygenic model [112]. The most recent GWAS on ER-negative breast cancer found that there were no significant associations with any of the variants examined and found no evidence to suggest that ER-negative breast cancer has a polygenic basis for disease development, unlike ER-positive breast cancer [149]. Thus, recent GWAS on breast cancer have reiterated that importance of viewing breast cancer sub-types as distinct entities and underpin the genetically diverse pathways by which these tumour sub-groups develop.

In conclusion, although the variants identified by GWAS have little clinical applicability at present, it is likely that more refined GWA studies of tumour sub-groups with defined pathological characteristics will shed more light on risk prediction in the future.

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