

REVIEW



Identifying breast cancer susceptibility genes – a review of the genetic background in familial breast cancer

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ABSTRACT

Introduction: Heritage is the most important risk factor for breast cancer. About 15–20% of breast cancer is familial, referring to affected women who have one or more first- or second-degree relatives with the disease. The heritable component in these families is substantial, especially in families with aggregation of breast cancer with low age at onset.

Identifying breast cancer susceptibility genes: Since the discovery of the highly penetrant autosomal dominant susceptibility genes *BRCA1* and *BRCA2* in the 1990s, several more breast cancer genes that confer a moderate to high risk of breast cancer have been identified. Furthermore, during the last decade, advances in genomic technologies have led to large scale genotyping in genome-wide association studies that have identified a considerable amount of common low penetrance loci. In total, the high risk genes, *BRCA1*, *BRCA2*, *TP53*, *STK11*, *CD1* and *PTEN* account for approximately 20% of the familial risk. Moderate risk variants account for up to 5% of the inherited familial risk. The more than 180 identified low-risk loci explain 18% of the familial risk. Altogether more than half of the genetic background in familial breast cancer remains unclear. Other genes and low risk loci that explain a part the remaining fraction will probably be identified.

Clinical aspects and future perspectives: Definitive clinical recommendations can be drawn only for carriers of germline variants in a limited number of high and moderate risk genes for which an association with breast cancer has been established. Future progress in evaluating previously identified breast cancer candidate variants and low risk loci as well as exploring new ones can play an important role in improving individual risk prediction in familial breast cancer.

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Introduction

Breast cancer is the most common cancer type affecting women, representing 25% of all cancer [1]. Many risk factors for breast cancer have been identified including hormonal and lifestyle factors, the most important one nevertheless being heritage (Table 1). The individual risk increases proportionally with the number of close relatives affected by breast cancer and early age at onset. The heritable component in familial clustering of breast cancer has been estimated to 73% and the environmental proportion 27%. Furthermore, familial breast cancer with low age at onset is mostly due to heritable factors [18]. Clustering of breast cancer in families has been investigated and supported by epidemiological studies since the late 1940s [19]. The search and identification of risk genes for hereditary breast cancer progressed in the early nineties when linkage analyses in affected families led to the discovery of *BRCA1*, *BRCA2*. A candidate gene approach using affected family members in cancer families led to the discovery of *TP53* as the causal factor behind Li-Fraumeni syndrome (LFS). These high penetrance traits and identification of the breast cancer predisposing genes *STK11*, *CDH1* and *PTEN*, that followed, have the occurrence of

breast cancer in common as a part of a cancer syndrome, with several types of cancer involved. About 5–10% of breast cancer are thought to be hereditary with 4–5% being due to already identified high penetrance traits inherited in an autosomal dominant inheritance fashion [20–24]. On the other hand, 15–20% of breast cancer is familial, referring to affected women who have one or more first- or second-degree relatives with the disease [7,9,20,25]. Further linkage and candidate gene studies have been unsuccessful in finding more high-penetrance susceptibility genes despite the fact that the already identified ones only represent a small part of the estimated total heritable fraction. It has become clear that the remaining fraction is not explained by variants in high-risk breast cancer genes. Evidence has accumulated that breast cancer is inherited as a polygenic disease where pathogenic variants with differential impact together confer an increased risk. Focus has shifted toward research of genetic risk factors that confer a low to moderate risk of breast cancer. The first identified moderate risk gene, cell cycle checkpoint kinase 2 (*CHEK2*) identified in 2002, was found to cause a twofold risk of breast cancer and an up to fourfold risk in familial breast cancer cases [26,27]. Advances in

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Table 1. Risk factors in breast cancer (adapted from Hulka and Stark [2]).

	Factor	Risk group	Effect on risk (RR)	References
Demographic factors	Geographic area	US and Northern Europe vs. Africa, Asia	3	GLOBOCAN [1]
Family history	On close relative with breast cancer	Yes vs. no	1.1–2	Pharoah et al. [3], Claus et al. [4]
Reproductive factors	Two first degree relatives	Yes vs. no	3.6	Pharoah et al. [3]
	Age at menarche	Early vs. late	1.1–2.0	Hulka and Stark [2]
	Age at menopause	Late vs. early	1.1–2.0	Hulka and Stark [2]
	Age at first birth	>30 vs. <20 years	1.1–2.0	Hulka and Stark [2]
	Parity	Nulliparous vs. multiparous	1.1–2.0	Hulka and Stark [2]
Other hormonal factors	Breastfeeding	None vs. >1 year	1.1–2.0	Lancet [5], Hulka and Stark [2]
	BMI (postmenopausal)	High vs. low	1.1–2.0	van den Brandt et al. [6]
	BMI (premenopausal)	Low vs. high	1.1–2.0	van den Brandt et al. [6]
	Oral contraceptives	Ever vs. never use	1.07	Lancet [7]
	Oral contraceptives	Current use vs. none	1.24	Lancet [8]
	HRT	Current use vs. none	1.1–2.0	Lancet [9]
	Serum estradiol	High vs. low	1.1–2.0	Thomas et al. [10], Hankinson et al. [11]
Breast properties	Mammographic density	Dense vs. not	4–5	McCormack and dos Santos Silva [12]
	Atypical ductal hyperplasia	Yes vs. no	1.1–2.0	Byrne et al. [13], Page et al. [14]
Other	Ionizing radiation to the chest at young age	Yes vs. no	1.1–2.0	Hancock et al. [15]
	Alcohol	Daily intake vs. none	1.2–2.0	Schatzkin and Longnecker [16], Smith-Warner et al. [17]

RR: relative risk.

genomic technologies have led to large scale genotyping in genome-wide association studies (GWAS) that have identified a considerable amount of common low penetrance loci. Great progress has been made, but a substantial portion of the genetic background in familial breast cancer is yet unexplained. In addition, the functional and clinical implications of some of the risk-genes already identified are limited since robust risk estimates are lacking and because their polygenic context is unclear. This review gives an overview of the genetic background behind familial breast cancer.

Identifying breast cancer susceptibility genes

Various approaches have led to the identification of breast cancer susceptibility genes with risk alleles that in general are divided into three groups depending on their frequency and conferred risk. The Human Genome Variation Society (HGVS) has developed an internationally accepted nomenclature that recommends the use of the neutral term variant rather than mutation. The risk variants mostly show an inversely proportional impact, from very rare ones with high penetrance to the common low risk single nucleotide variants with allele frequency of up to 50%:

- *High-risk variants:* Very rare in the population with a minor allele frequency of <0.005. The conferred relative risk of breast cancer is higher than 4.
- *Moderate-risk variants:* Rare, with a minor allele frequency of 0.005–0.01. Pathogenic variants confer a relative risk of 2–4.
- *Low-risk variants:* Minor allele frequency >0.05 and conferred risk of breast cancer of less than 1.5-fold [28].

High-risk genes

The genes underlying high-risk syndromes like *BRCA1* and *BRCA2* have mainly been identified through linkage analysis

and positional cloning. When Mary-Claire King and her team began the work on identifying *BRCA1*, epidemiological studies had already demonstrated that the risk of breast cancer was increased in first degree relatives of affected women, especially if they were young at onset and/or had bilateral disease [29]. Mechanisms such as polygenetic effects and/or shared environmental factors could possibly explain the familial clustering of breast cancer. However, a segregation analysis in a large sample of families provided evidence that familial clustering of breast cancer could be explained by a highly penetrant autosomal dominant susceptibility gene in 4% of the families [22]. The linkage analysis was performed in 23 families with a severe family history of breast cancer and thus eliminating the risk of including cases of sporadic breast cancer. In 1990, after 17 years of research, the results of the linkage analysis were published, a high-risk gene assigned to chromosome 17q21 had been identified [30]. The locus on chromosome 17 was immediately confirmed by Narod et al. and, in addition, an association with ovarian cancer was established [31]. Subsequently, in 1994, the *BRCA1* gene was identified through positional cloning and truncating mutations in the protein coding sequence were found to segregate with the disease [32]. In 1994, an international team lead by Stratton et al. analyzed 15 families, with early onset breast cancer that were not caused by *BRCA1*, to search for a second high-risk gene that was localized to chromosome 13q12-13 [33]. Shortly thereafter *BRCA2* was cloned. Tumour samples were examined for loss of heterozygosity and an association with defect *BRCA2* was confirmed [34,35]. Li-Fraumeni syndrome was first described in 1969 as a rare clinical syndrome with a high penetrance of premenopausal breast cancer and sarcomas, brain tumors and adrenal cortical carcinomas at very young age [36]. The rarity and high mortality of the syndrome made linkage analysis difficult. Instead a candidate gene approach was used to identify germline mutations of the tumor suppressor gene *TP53*,

located on chromosome 17p13.1, as the causative factor. *TP53* was selected as a plausible gene because it was identified as the most common inactivated gene in sporadic forms of cancers associated with Li-Fraumeni [37,38].

Moderate-risk genes

Also the first identified moderate risk gene, *CHEK2* (1100delC), was localized through linkage-analysis of a large non-*BRCA* family [26]. To assess the conferred risk, linkage analysis was combined with an association study since moderate risk genes display incomplete co-segregation due to their intermediate penetrance. For that reason, candidate gene approach is more applicable when identifying moderate penetrance genes. This search has focused on candidate genes, which like *BRCA1* and *BRCA2*, are involved in DNA damage response or other pathways important for breast cancer biology. By resequencing genes in cases from highly penetrant families, moderate susceptibility genes have been identified and thereafter evaluated in case-control association studies. Breast cancer genes identified with this approach include pathogenic variants in moderate risk genes *ATM* [39–41] and *PALB2* [42,43]. Further studies of loss-of-function variants of the *PALB2* gene suggest that the associated breast cancer risk in carriers may overlap with that for the high-risk category [44]. Since the high-throughput technique next generation sequencing (NGS), now more affordable whole-exome sequencing has become a common approach to identify rare variants. When searching for rare risk alleles, reduced statistical power demands sequencing of enormous numbers of unselected cases and controls. One strategy to reduce sample size is by performing a staged study starting with sequencing in a small cohort of cases with familial aggregation of breast cancer, assuming that genetic contribution is higher. The cases can also be chosen due to certain criteria such as being negative for *BRCA1* and *BRCA2*, tumor phenotype or sharing a previously identified susceptibility gene. Identified risk variants are thereafter genotyped in larger cohorts. Pathogenic variants in a new breast cancer susceptibility gene, *RECQL*, were identified through whole-exome sequencing in two cohorts consisting of 144 Polish and 51 French-Canadian women with familial breast cancer who were all negative for *BRCA1/2* founder mutations. In the discovery phase, candidate genes for further study were selected by focusing on highly deleterious mutations in genes with a function previously associated with cancer. Several truncating mutations in the gene *RECQL*, a DNA-helicase gene previously related to cancer, were identified. Pathogenic *RECQL* variants were then validated in larger cohorts containing patients with sporadic and familial breast cancer as well as a control group [45].

Common low-risk variants

The results of searches for the third category of susceptibility genes, common low risk variants, have been mainly achieved through GWAS. This approach scans most of the genome for causal genetic variants without any assumption of biological

function or location. The genome of two individuals is 99.55% identical, but the variations can greatly affect the risk of various complex diseases. More than 10 million of the single nucleotide polymorphisms (SNPs), with a minor allele frequency of 1% or more, are collected in a database, dbSNP [46]. Sets of SNPs in a genomic region are in strong linkage disequilibrium (LD) inherited in blocks. These blocks contain a large number of SNPs with specific patterns, or haplotypes, that can be identified with a reduced number of tag-SNPs. All tag-SNPs have been categorized through the HapMap project. Due to the high correlation between the variants (high LD), a few hundred thousand of SNPs can be used as markers over the whole genome. In GWAS, genotyping platforms that allow evaluation of hundreds to thousands of SNPs simultaneously, are utilized. In general, in a first phase of a GWAS, tag-SNPs are genotyped in a smaller number of cases and controls. In the second and third phase, variants with the strongest evidence are narrowed down to a small number and replicated through testing for significance in large association studies. The identified low-risk variants are not always the causal ones; any SNPs within the haplotype block could play the role of casual SNP. Fine mapping, that contains genotyping of all common variants of risk loci, can bring more clarity to the issue. Furthermore, most of the identified variants are located in non-coding regions, intergenic and intronic variants are common. They may have an effect on the regulation of gene expression or function. So far more than 180 low risk loci associated with breast cancer risk have been identified [47–70]. Since it is more affordable and less complicated than complete resequencing of whole genomes, candidate gene-association studies have also been used to search for common low-penetrance alleles, but with limited progress.

Evidence of association for specific genes and variants

The following section is an overview of genes in which evidence of an association with breast cancer has been established, their function, conferred risk of breast cancer and other associated tumors. Most disease-associated variants with a moderate to high risk of breast cancer are protein truncating. These are nonsense mutations, frame-shift insertions and deletions and splice site affecting variants that in general cause a premature stop codon and as a result, a nonfunctioning protein product. Some of the alterations associated with breast cancer risk are missense variants that refer to single base pair substitutions causing a change of one amino acid. Compared to truncating variants, the effect of a missense variant is more difficult to assess, the amino-acid change may not have a dramatic effect on the resulting protein. Larger structural genomic rearrangements, including deletions and duplications of regions, can also cause gene inactivation. Silent mutations do not alter amino acids and are in general considered functionally irrelevant, but may be oncogenic by altering transcript splicing. To facilitate the clinical interpretation of variants, the HGVS recommends the use of a five category functional classification system: (1) benign variant; (2) likely benign variant; (3) variant of uncertain significance; (4) likely

disease-associated variant; (5) disease-associated variant. Variants of unknown significance are often missense variants. Risk assessment of the pathogenicity of missense variants can be time-consuming and requires analyses of whether the particular variant affects an evolutionary conserved region, the probable impact on protein function as well as genetic analyses in affected families and tumor subtypes. Family segregation analysis can sometimes yield data to re-classify a variant of uncertain significance. Disease-associated missense variants are in general associated with a lower risk than truncating variants. For instance, the *CHEK2* truncating variant *c.1100delC* is associated with a higher risk of breast cancer compared to *CHEK2* missense variant *I157T* (*c.470A > G*) [71]. One exception is that the majority of *TP53* mutations are highly penetrant missense variants. These variants are referred to as dominant negative since the altered *TP53* protein causes inactivation of wild-type *TP53* protein [72].

Risk estimates for the established breast cancer susceptibility genes are in general not completely reliable due to ascertainment bias (especially for *TP53*, *STK11* and *PTEN*). Many studies have been based on families with high risk features and, as a result, breast cancer risk may be overestimated in carriers with less pronounced family history. In comparison, population-based studies mostly have generated lower risk estimates [24,73,74].

BRCA1 and BRCA2

The *BRCA1* gene encodes a multi-domain protein that functions in a number of cellular pathways to maintain genomic stability, including cell cycle checkpoint activation as well as transcriptional regulation and apoptosis [75]. *BRCA1* and *BRCA2* are also important for DNA repair; specifically in homologous recombination (HR) of double-strand DNA breaks (DSB) [75]. *BRCA1* and *BRCA2* mutations confer a very high life-time risk of breast cancer in the range of 50–80% for *BRCA1* whereas the risk of ovarian cancer is 30–60% for *BRCA1* but slightly lower for *BRCA2* carriers, that of 10–25% [76]. The relative risk of breast cancer has been estimated to 11.4 for *BRCA1* and 11.7 for *BRCA2* [77]. *BRCA1* and *BRCA2* pathogenic variants are found almost throughout the genes whole coding sequence and are usually truncating. Pathogenic missense variants have also been described in functional domains. In addition, different pathogenic variants show a varying spectrum of allelic effects such as the missense variant, R1699Q (*c.5096G > A* p.Arg1699Gln) in *BRCA1*, associated with a significant increased risk of breast cancer, but much lower than the average risk for carriers in general. The truncating variant, p.Lys3326 (rs11571833) in *BRCA2* is considered to be a low-risk allele [78,79].

Based on gene expression data, breast cancer tumors are characterized and divided into four subtypes (luminal A, luminal B, HER2-enriched and basal-like). Most *BRCA1*-related breast cancers have a basal-like phenotype and they are also characterized by the lack of expression of estrogen-receptors, progesterone-receptors and of no overexpression of human epidermal growth factor 2 (triple negative breast cancer). In addition, over-expression of the epidermal growth factor

receptor (EGFR) has been associated with *BRCA1*-related breast cancers [80]. The immunophenotype and gene expression profile of *BRCA2*-related cancers are very similar to sporadic breast cancers. Both *BRCA1* and *BRCA2* tumors exhibit a higher histological grade, *BRCA1* tumors are more often poorly differentiated (grade 3) whereas *BRCA2* tumors more frequently are moderately or poorly differentiated (grades 2 and 3) [81]. The majority of *BRCA1* and *BRCA2*-associated ovarian cancers are classified as high-grade serous carcinomas (HGSC) [82]. There is growing evidence that HGSC originates in the fallopian tubes. This has been discovered since intraepithelial or early invasive carcinomas of the fallopian tube frequently are identified in *BRCA* mutation carriers who undergo prophylactic risk-reducing salpingo-oophorectomy [83].

Studies of other tumor types associated with *BRCA1* have been inconclusive [84,85]. Besides breast and ovarian cancer in women, tumors linked with *BRCA2* mutations include pancreatic and early onset prostate cancer. For *BRCA2* carriers, the risk of male breast cancer before 80 years has been estimated to 7% [86].

TP53

One of the most intensively studied tumor suppressors is tumor protein 53 (*TP53*) since loss of wild-type *TP53* activity is frequently detected in many different tumor types. The protein regulates the cell cycle, interacts in DNA repair, apoptosis, cellular senescence and metabolism. Inherited *TP53* mutations are associated with the rare autosomal dominant disorder, the LFS. Female mutation carriers have a nearly 100% lifetime risk of cancer compared to 73% for males, the difference being caused by breast cancer [87]. More studies have found the same levels of cancer risk in carriers [88,89]. Breast cancer is the most common tumor with a 49% risk of being affected before 60 years, but most women are diagnosed before age 40 [90]. The increased risk of breast cancer for disease-associated variants has been reported to be >100-fold (age-adjusted relative risk 105CI 62–165) [77].

Unlike other high-risk genes that mostly display a risk associated with truncating mutations, genotype–phenotype analysis in LFS families has revealed that germline missense mutations are more frequent. A majority of these mutations are missense variants in the DNA-binding domain. In addition, missense variants have been associated with earlier onset, compared to truncating variants, due to a dominant negative effect that interfere wild-type p53 function [72]. There is also a more than 20% frequency of *de novo* mutations [87]. Other than breast cancer in women, *TP53* mutation carriers are at increased risk of early-onset and multiple primary cancers including sarcomas, brain and adrenocortical tumors [91]. Lymphoma, leukemia, melanoma, lung, pancreas, prostate and ovarian cancers also seem to be more frequent. Childhood-onset tumors exists the most common are brain tumors, followed by sarcomas [91–93].

PTEN

Cowden syndrome is rare condition caused by germline mutations in tumor suppressor gene *PTEN* [94]. *PTEN* encodes a multifunctional protein with roles in the PI3K/AKT-mTOR signaling pathway. Studies of carriers of disease-causing variants show a considerably high lifetime risk of breast cancer, with low age of onset. Carriers are also at an increased risk of several other malignancies, especially thyroid and endometrial cancer [95–97]. The syndrome is otherwise characterized by multiple hamartomas of the gastrointestinal tract, macrocephaly and benign tumors such as lipomas.

STK11

The tumor suppressor *STK11* is another gene with a gene product important for cell cycle regulation and mediation of apoptosis. Deleterious mutations cause Peutz–Jeghers syndrome characterized by intestinal hamartomatous polyps and mucocutaneous pigmentation [98]. In addition, the lifetime risk of breast cancer by 60 years has been reported to be 32–54% [99]. Other associated tumors with markedly elevated risk are cancers of gastrointestinal origin and pancreatic cancer. Female carriers are also at an increased risk of ovarian sex cord-stromal tumors and a rare tumor of the cervix, adenoma malignum. Carriers of *STK11* mutations have a cumulative lifetime risk of any cancer of up to 85% [99].

CDH1

The *CDH1* gene encodes a protein responsible for cell-to-cell adhesion and functions as a cell invasion suppressor [100]. E-cadherin germline mutations are responsible for hereditary diffuse gastric cancer (HDGC). Carriers of truncating variants are at a very high risk of diffuse gastric carcinoma at young age and, in addition, an estimated relative risk of breast cancer of 6.6 (predominantly lobular breast cancer) [101]. Recent studies have provided evidence of lobular breast cancer as the first manifestation of HDGC. Deleterious *CDH1* mutations have been identified in women with bilateral lobular breast cancer without a family history of diffuse gastric cancer. Also novel deleterious *CDH1* alterations have been identified raising the question whether lobular breast cancer can be inherited as an independent E-cadherin syndrome [102,103].

PALB2

The *PALB2* gene encodes a protein that plays an important role in maintaining genomic integrity through its role in HR and DNA repair. Originally identified as a *BRCA2*-interacting protein, *PALB2* has been shown to be crucial for key *BRCA2* tumor suppressor functions [104]. *PALB2* was first associated with breast cancer as a risk gene with moderate penetrance in 2007. Loss-of-function *PALB2* mutations have however in a meta-analysis yielded a combined estimated relative risk of 5.3 in carriers of pathogenic mutations which suggests that *PALB2* should possibly instead be placed in the high-risk category [77]. A predisposition for ovarian- and pancreatic

cancer conferred by *PALB2* mutations has been indicated, though larger studies are needed to address this question [43,105,106].

CHEK2

Checkpoint kinase 2 is in response to DSB or replicative stress activated by *ATM* [107]. Activated *CHEK2* plays an important role in phosphorylating downstream substrates including tumor suppressors *TP53* and *BRCA1* [108]. Originally *CHEK2* was found mutated in Li-Fraumeni patients and one of the truncating mutations c.1100delC, has been reproducibly associated with breast cancer risk in different populations. This truncating variant seems to be more frequent in populations in parts of Northern and Eastern Europe compared to Southern Europe and North America [109,110]. Besides the *CHEK2**1100delC variant, two more truncating variants have been associated with similar breast cancer risk (del5395 and IVS2+1G>A) whereas one missense mutation, I157T (c.470A>G) confers only slightly increased risk [71,111,112]. Recently, a large collaborative study, using data from the Breast Cancer Association Consortium (BCAC), reported a relative risk of breast cancer of 2.26 in *CHEK2**1100delC carriers. The risk of breast cancer associated with *CHEK2**1100delC is though markedly higher in carriers with a family history of breast cancer than without which complicates risk prediction. Carriers without a family history may have a life-time risk close to the average population risk and consequently it has been suggested that family history should be taken into account in risk prediction of healthy carriers [110]. A 3.5-fold excess risk of *CHEK2**1100delC carriers to develop contralateral breast cancer has been found as well as a worse prognosis compared to non-carriers, especially for estrogen receptor (ER)-positive breast cancer [113,114]. There have been reports of an association between *CHEK2* pathogenic variants and ovarian cancer and several other additional malignancies, but the results have been inconclusive [115,116]. A meta-analysis has provided evidence that *CHEK2**1100delC carriers may be at increased risk of colorectal cancer [117].

NBN

The *MRE11A-RAD50-NBN* (*NBS1*) is an evolutionary conserved protein complex (the MRN complex) important for detection and early processing of double strand breaks. Carriers of heterozygous *NBN* mutations have a significant increased risk of breast cancer with an estimated odds ratio of 3.1 [118]. In a meta-analysis of seven studies, strong evidence was presented for a moderately increased breast cancer-risk associated with a truncating variant, c.657del5 with a relative risk of 2.7 [119]. Another study provided evidence for an association between the variant and early onset breast cancer [120].

ATM

Homozygous or compound mutations in the ataxia-telangiectasia mutated gene (*ATM*) cause a neurodegenerative disorder characterized by cerebellar ataxia, immunological deficiency, hypersensitivity to ionizing radiation and

increased risk of cancer. The gene encodes a protein kinase involved in DNA repair of double strand breaks through downstream interaction with *TP53*, *BRCA1* and *CHEK2* proteins in the damage response pathway [121]. Furthermore, the MRN-complex recruits *ATM* protein and initiates phosphorylation of substrates in response to DNA-damage [122]. A meta-analysis found heterozygous carriers of *ATM* mutations at an estimated relative risk of breast cancer of 2.8 [77]. The *ATM* missense variant p.Val2424Gly (c.7271T>G) has been associated with a higher risk compared to truncating variants and is another example of a dominant negative mutation [123]. An increased risk of pancreatic cancer has been indicated in carriers of pathogenic *ATM* variants [124].

NF1

The protein product of the Neurofibromin 1 gene (*NF1*) acts as a tumor suppressor by negative regulation of the Ras signal transduction pathway [125]. Pathogenic variants in the *NF1* gene cause Neurofibromatosis type 1, an autosomal dominant cancer syndrome, with a very variable phenotype, characterized by skin pigmentation and tumors of the nervous system. A Finnish study with a population-based cohort found a 59.6% lifetime risk of cancer. Excess risk of breast cancer was moderate, but highest in women younger than 40 years [126]. These results were supported in two more studies of *NF1* associated breast cancer risk that found an increased risk in women less than 50 years, but thereafter incidence patterns similar to the general population. The relative risk of breast cancer in carriers was estimated to 2.6 [77,127,128].

Other breast cancer gene susceptibility candidates

The fact that *BRCA1*, *BRCA2* and *PALB2* function in the repair of DNA double-strand breaks has promoted further studies on candidates in pathways controlling DNA integrity. Several other susceptibility genes with protein products involved in DNA damage signaling and repair have in different populations been associated with breast cancer risk in single studies, but have not been confirmed in large systematic associations studies. Most of the identified pathogenic variants are very rare, which makes a putative association with breast cancer and a robust estimate of penetrance difficult to assess. *RAD51*, a key protein in mediating HR, forms a complex with a family of accessory proteins known as *RAD51* paralogs. Deleterious mutations in *RAD51* paralogs *RAD51C*, *RAD51D*, have shown clear evidence of an association with ovarian cancer, but there is insufficient evidence for an association with increased risk of breast cancer [129,130]. Common low risk variants in *RAD51B* have been associated with familial breast cancer and male breast cancer [131]. Exome sequencing and a subsequent association study identified *RAD51* paralog, *XR2CC*, as a putative breast cancer gene [132]. *BRIP1*, that encodes a DNA helicase, has also shown clear evidence of predisposing for ovarian cancer but a role in breast cancer risk has not found support [133]. A recent large-scale association study could not provide evidence for an association between any truncating variant in

the *BRIP1* gene and breast cancer [134,135]. The statistical analysis allowed exclusion of a twofold risk of breast cancer, which in general is considered the lower threshold for a moderate susceptibility gene. Recently, rare mutations in two other helicase genes, *RECQL* and *BLM* have been associated with breast cancer [45,136]. Biallelic mutations in *BRIP1* as well as *PALB2*, *RAD51C* and *BRCA2* (also known as the *FANCI*, *FANCD1*, *FANCN* and *FANCO* genes) cause Fanconi Anemia (FA), which has prompted a search of other FA genes predisposing for breast cancer. Exome sequencing has identified heterozygous truncating mutations in FA genes *FANCC* and also in *FANCM* with a particularly strong predisposition for triple negative breast cancer [136–138]. A limited number of pathogenic germline mutations in the *MRE11* and *RAD50* have also been suggested as breast cancer predisposing variants, but their role is yet to be determined [139].

Low risk variants associated with breast cancer

The BCAC, a collaboration with more than 50 participating breast cancer case-controls studies, has conducted several combined large-scale genotyping studies. In a BCAC study from 2015, a GWAS of more than 120,000 cases and controls added 15 more low risk loci to the previously more than 90 identified [47–69,79]. The most recent genome wide association study, performed by BCAC and the OncoArray Consortium, added 65 more loci associated with breast cancer risk, 19 of them more strongly associated with ER-positive disease. Furthermore, it was estimated that in total common low risk variants identified by GWAS explain 18% of the familial relative risk [70]. Most of the identified variants are intergenic or intronic with uncertain function, but they may be important for gene expression and function.

Only a few causal protein coding variants have been suggested, one being the SNP rs11552449 which is a missense substitution in the *DCLRE1B* gene (*SNM1B*) involved in DNA cross-link repair [58]. Further investigation of a tag SNP in an intron of the *EXO1* gene, with a gene-product involved in mismatch repair, found a strong association with a putative causal variant in the coding region of the gene. The associated missense variant, rs4149909, encodes an amino acid substitution, with potential pathogenic effect related to breast cancer [69]. Several GWAS have investigated specific effects by breast cancer subtypes, mainly by ER status. Analysis of 74 breast cancer SNPs in the subgroup of triple negative breast cancer found that 24 of the 74 SNPs were also individually associated with triple negative breast cancer [140]. A GWAS in about 40,000 cases of ER-negative breast cancer and *BRCA1* mutations carriers identified 10 new variants associated with ER-negative breast cancer. Furthermore, previously identified variants were replicated and altogether the 125 associated variants were estimated to explain 16% of the familial risk of ER-negative breast cancer [141].

Genetic contribution in familial breast cancer

Overall, *BRCA1* and *BRCA2* mutations are estimated to be responsible for about 3% and the other less common high

penetrance genes account for less than 1% of all breast cancer [23,24]. However, a recent study though suggests that *BRCA1* and *BRCA2* account for only 1.4% of all breast cancer and that other results with higher proportions could be due to founder populations or to patient selection [142]. In Sweden, carrier frequency of *BRCA1* was less than 1% in an unselected breast cancer cohort in Stockholm County [143]. Pathogenic variants in *BRCA1* and *BRCA2* account for 16% of the genetic contribution in familial breast cancer risk [144]. In total, the high risk genes, *BRCA1*, *BRCA2*, *TP53*, *STK11*, *CD1* and *PTEN* account for approximately 20% of the familial risk [23,145,146]. Moderate penetrance variants account for up to 5% of the inherited familial risk [23,147]. The more than 180 identified low-risk loci explain 18% of the familial risk [70]. Altogether more than half of the genetic background in familial breast cancer is still unclear (Figure 1).

Genetic modifiers

Evidence has accumulated that familial breast cancer is a polygenic disease and that the genetic susceptibility is driven by a large number of variants with multiplicative effects on breast cancer risk [148,149]. This multiplicative model could explain a part of the residual component also in carriers of high-risk genes such as *BRCA1* and *BRCA2*. Several studies have found support for low-risk variants that modify risk in *BRCA1* and *BRCA2* mutations carriers [150–153]. There is a markedly higher

lifetime risk, in *CHEK2*1100delC* carriers, with a family history of breast cancer compared to those without, which suggests that *CHEK2*1100delC* combines with other genetic variants that together modifies the risk of breast cancer [114]. A recent study investigated whether the multiplicative model was applicable on *CHEK2*1100delC* and common low penetrance susceptibility variants previously associated with breast cancer risk. A polygenic risk score (PRS) was calculated based on the effect of 77 low penetrance variants. The synergistic effect of *CHEK2*1100delC* and the PRS on breast cancer risk was estimated and the result provided strong support for a multiplicative polygenic model. The effect of the PRS was slightly attenuated when adjusting for family history, but remained an independent risk factor. The PRS was proposed as a tool for stratifying risk in *CHEK2*1100delC* carriers [154]. It is not clear whether the model can be applicable on combinations of moderate to high-penetrance genes. A study of the combined effects of *BRCA*, *ATM* and *CHEK2* mutations, found no support for a multiplicative effect on breast cancer risk. This may reflect their biological interaction in DNA-repair. Impairment of functions up-stream of *BRCA*, such as *ATM* and *CHEK2*, may confer little or no additional risk of breast cancer [155]. Investigating this issue is difficult since carriers of any combinations of high and moderate penetrance genes are very rare.

Breast cancer syndromes

A significant feature of the high penetrance breast cancer genes is the conferred risk also for other tumor types in the context of breast cancer syndromes such as the hereditary breast and ovarian cancer syndrome (HBOC) caused by deleterious variants in *BRCA1* and *BRCA2* (Table 2). A putative association with other types of cancer can be difficult to assess especially for rare risk genes with an intermediate penetrance. Regional variation of allelic variants is another compounding factor. An increased risk of other malignancies within families carrying disease associated variants in *CHEK2* includes colon, prostate, kidney and thyroid cancer, but only the association with colon cancer (*CHEK2*1100delC*) has been

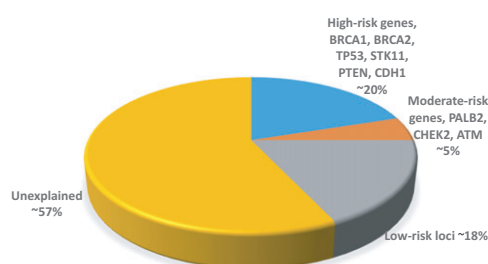


Figure 1. Fraction of the excess familial risk of breast cancer explained by the currently known susceptibility genes/loci.

Table 2. Genes for which an association between disease-associated variants and breast cancer has been established (modified from Easton et al. [77]).

Gene	Average relative risk ^a	References	Other cancer risks
<i>BRCA1</i>	11.4	Easton et al. [77]	Ovary (HBOC)
<i>BRCA2</i>	11.7	Easton et al. [77]	Ovary, prostate, pancreas (HBOC)
<i>TP53</i> ^c	105 (90% CI 62–165)	Hwang et al. [90]	Childhood sarcoma, adrenocortical carcinoma, brain tumors (Li-Fraumeni Syndrome).
<i>PTEN</i>	No reliable estimate ^d	Bubien et al. [96]	Thyroid, endometrial cancer (PTEN Hamartoma Tumour Syndrome PHTS, Cowden syndrome)
<i>CDH1</i>	6.6 (90% CI 2.2–19.9)	Tan et al. [97]	Diffuse gastric cancer (HDGC)
<i>STK11</i>	No reliable estimate ^d	Pharoah et al. [101]	Colon, pancreas, ovarian sex cord-stromal tumors (Peutz–Jeghers syndrome)
<i>PALB2</i>	5.3 (90% CI 3.0–9.4)	Hearle et al. [99]	Unknown or insufficient evidence
<i>NF1</i>	2.6 (90% CI 2.1–3.2)	Easton et al. [77]	Malignant tumors of peripheral nerve sheath, brain, central nervous system
<i>ATM</i>	2.8 (90% CI 2.2–3.7)	Easton et al. [77]	Unknown or insufficient evidence
<i>CHEK2</i>	2.26 (90% CI 1.9–2.69) ^b	Schmidt et al. [110]	Colon
<i>NBN</i>	2.7 (90% CI 1.9–3.7) ^e	Zhang et al. [119]	Unknown

^aRisk estimates for *BRCA1* and *BRCA2* were obtained from the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (Boadicea), from a single study for *TP53* and *CD1*, and from meta-analysis of multiple studies for the other genes [77]. Note that there is evidence that relative risk declines with age for carriers of mutations in *BRCA1* [156], *CHEK2* [157] and *ATM* [158]. The evidence for *PALB2* is weaker [44]. These represent average relative risks for all ages and may therefore underestimate the relative risk of breast cancer for younger persons and overestimate the relative risk for older persons. The estimates relate for protein-truncating variants except as noted.

^bMost data are for the *CHEK2*1100delC* variant.

^cMost pathogenic mutations in *TP53* are missense variants. Most published risk estimates are subject to ascertainment bias.

^dPublished risk estimates are subject to ascertainment bias.

^eRisk estimate for *NBN* c.657del5 variant.

supported in a meta-analysis [116,117]. Female carriers of variants associated with Lynch syndrome are at increased risk of ovarian and endometrial cancer, in addition to an increased risk of gastrointestinal and urinary tract tumors [159]. Allelic variants in one gene behind the syndrome, *MLH1*, has also been associated with increased risk of breast cancer (18.6% cumulative risk to age 70 years 95% CI, 11.3–25.9), but evidence published so far has not been considered sufficiently robust enough to make specific recommendations for screening programs for carriers [160]. *BAP1* tumor predisposition syndrome (*BAP1*-TPDS) is characterized by early onset benign melanocytic skin tumors and, later in life, mesothelioma and uveal melanoma. An increased risk of breast cancer has been indicated, but not confirmed, in carriers of germline mutations in *BAP1* [161]. Germline *CDKN2A* mutations have been found to predispose to high penetrance familial melanoma. Carriers also have a markedly increased risk of pancreatic cancer. In addition, a moderate risk of breast cancer has been suggested [162].

Other studies have focused on investigating familial breast cancer and any putative associations with other tumor types in families without any previously identified disease associated variants. In a study of 803 Swedish families with hereditary breast cancer tumors in the colon, ovary, endometrium, pancreas and liver, as well as leukemia were overrepresented. A putative breast and endometrial cancer syndrome was suggested [163]. An association between breast- and endometrial cancer was supported again by a study of tumor spectrum in non-*BRCA* breast cancer [164]. A recently published study based on data from the Swedish Family-Cancer Database calculated relative risk for other types of tumors in families with aggregation of breast cancer. The strongest associations with breast cancer were found for prostate and ovarian cancer (p value $<10^{-11}$). Other associated tumor types were for example stomach and male colorectal cancer ($p < 2.5 \times 10^{-6}$), pancreatic cancer ($p < 5 \times 10^{-4}$), thyroid, endometrial and testicular cancer ($p < .0025$) [25].

Clinical aspects and future perspectives

Today, definitive clinical recommendations can be drawn only for carriers of germline mutations in the genes given in Table 2. These genes have been found to have a strong association with increased risk of breast cancer. *BRCA1* and *BRCA2* are considered high risk genes, even though risk estimates, could be slightly overestimated due to ascertainment bias. Furthermore, disease associated variants in *TP53*, *STK11*, *CDH1* and *PTEN* appear to confer high risks of breast cancer, but risk estimates are uncertain and biased by oversampling of carriers with a family history of breast cancer. Also *PALB2* may fall into the high-risk group, but confidence interval for the risk estimate is wide which makes risk level uncertain. Genes that are placed in the moderate risk category are *CHEK2*, *NF1*, *NBN* and *ATM*.

However, the risk associated with many missense variants in the established genes seems lower than for truncating variants whereas the risk from many other missense variants is still unclear. International collaborations could possibly

provide reliable estimates of risk by performing studies in large series of patients from diverse populations. Combinations of low risk variants are likely to be incorporated in testing panels, but need validation. Further studies of low risk variants but also candidates breast cancer genes are therefore essential. Prospective well designed studies of combinations of low penetrance variants with or without the presence of a high- or moderate risk variant could provide evidence for decisive risk estimates. Another issue that needs clarification is how different risk factors interact. Risk assessment in women at an increased risk of breast cancer is in general limited due to insufficient knowledge of how a given variant impacts in combination with other genetic risk factors, life-style factors and family history in terms of an absolute risk.

Previously identified disease-associated variants account for less than half of the inherited risk behind breast cancer, the rest remains unclear. Linkage analysis, in families at high risk of breast cancer, has not been able to find additional high penetrance genes which suggests that, if they exist, they probably account for only marginal fraction of the familial risk. Unidentified low risk variants could explain a part of the residual inherited risk [70]. Previously unidentified rare breast cancer genes could also contribute to the increased risk of breast cancer in these families. Further GWAS in large series of patients from different populations will continue to play an important role, but candidate gene studies are also of great importance, especially since the results seem to overlap only to a small extent [165]. Several studies show that breast cancer is associated with other tumor types which suggests that they could also share genetic susceptibility similarly to the previously identified breast cancer syndromes [25,163,164]. One approach in the search for new risk variants can therefore be to target these families with cases of breast cancer and other associated tumor types since the association implies that they may share genetic susceptibility.

To summarize, testing with gene panels is essential to identify women at a high risk of breast cancer, although the genes with established association with increased risk of breast cancer explain only a limited part of the inherited risk. Future progress in evaluating previously identified breast cancer candidate variants and exploring new ones can play an important role in improving individual risk prediction in familial breast cancer.

Disclosure statement

The authors report no conflicts of interest.

References

- [1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87–108.
- [2] Hulka BS, Stark AT. Breast cancer: cause and prevention. *Lancet.* 1995;346:883–887.
- [3] Pharoah PD, Day NE, Duffy S, et al. Family history and the risk of breast cancer: a systematic review and meta-analysis. *Int J Cancer.* 1997;71:800–809.

- [4] Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. *Cancer*. 1994;73:643–651.
- [5] Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet*. 2002;360:187–195.
- [6] van den Brandt PA, Spiegelman D, Yaun SS, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *Am J Epidemiol*. 2000;152:514–527.
- [7] Claus EB, Schildkraut JM, Thompson WD, et al. The genetic attributable risk of breast and ovarian cancer. *Cancer*. 1996;77:2318–2324.
- [8] Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet*. 1996;347:1713–1727.
- [9] Whittemore AS, Gong G, Itynre J. Prevalence and contribution of BRCA1 mutations in breast cancer and ovarian cancer: results from three U.S. population-based case-control studies of ovarian cancer. *Am J Hum Genet*. 1997;60:496–504.
- [10] Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. *Cancer Causes Control: CCC*. 1997;8:922–928.
- [11] Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst*. 1998;90:1292–1299.
- [12] McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2006;15:1159–1169.
- [13] Byrne C, Schairer C, Brinton LA, et al. Effects of mammographic density and benign breast disease on breast cancer risk (United States). *Cancer Causes Control: CCC*. 2001;12:103–110.
- [14] Page DL, Schuyler PA, Dupont WD, et al. Atypical lobular hyperplasia as a unilateral predictor of breast cancer risk: a retrospective cohort study. *Lancet*. 2003;361:125–129.
- [15] Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. *JNCI: J Natl Cancer Inst*. 1993;85:25–31.
- [16] Schatzkin A, Longnecker MP. Alcohol and breast cancer. Where are we now and where do we go from here? *Cancer*. 1994;74:1101–1110.
- [17] Smith-Warner SA, Spiegelman D, Yaun SS, et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA*. 1998;279:535–540.
- [18] Couto E, Hemminki K. Estimates of heritable and environmental components of familial breast cancer using family history information. *Br J Cancer*. 2007;96:1740–1742.
- [19] Smithers DW. Family histories of 459 patients with cancer of the breast. *Br J Cancer*. 1948;2:163–167.
- [20] Pharoah PD, Antoniou A, Bobrow M, et al. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet*. 2002;31:33–36.
- [21] Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet*. 1991;48:232–242.
- [22] Newman B, Austin MA, Lee M, et al. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. *Proc Natl Acad Sci*. 1988;85:3044–3048.
- [23] Ghousaini M, Pharoah PD. Polygenic susceptibility to breast cancer: current state-of-the-art. *Fut Oncol (London, England)*. 2009;5:689–701.
- [24] Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. *Anglian Breast Cancer Study Group. Br J Cancer*. 2000;83:1301–1308.
- [25] Zheng G, Yu H, Hemminki A, et al. Familial associations of female breast cancer with other cancers. *Int J Cancer*. 2017;141(11):2253–2259.
- [26] Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet*. 2002;31:55–59.
- [27] Vahteristo P, Bartkova J, Eerola H, et al. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. *Am J Hum Genet*. 2002;71:432–438.
- [28] Mavaddat N, Antoniou AC, Easton DF, et al. Genetic susceptibility to breast cancer. *Mol Oncol*. 2010;4:174–191.
- [29] Anderson DE. A genetic study of human breast cancer. *JNCI: J Natl Cancer Inst*. 1972;48:1029–1034.
- [30] Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science*. 1990;250:1684–1689.
- [31] Narod SA, Feunteun J, Lynch HT, et al. Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet*. 1991;338:82–83.
- [32] Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science (New York, NY)*. 1994;266:66–71.
- [33] Stratton MR, Ford D, Neuhausen S, et al. Familial male breast cancer is not linked to the BRCA1 locus on chromosome 17q. *Nat Genet*. 1994;7:103–107.
- [34] Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. *Nature*. 1995;378:789–792.
- [35] Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science*. 1994;265:2088–2090.
- [36] Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. *Ann Intern Med*. 1969;71:747–752.
- [37] Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science (New York, NY)*. 1990;250:1233–1238.
- [38] Srivastava S, Zou ZQ, Pirolo K, et al. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature*. 1990;348:747.
- [39] Easton DF. Cancer risks in A-T heterozygotes. *Int J Radiat Biol*. 1994;66:S177–S182.
- [40] Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet*. 2006;38:873–875.
- [41] Savitsky K, Bar-Shira A, Gilad S, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science*. 1995;268:1749–1753.
- [42] Rahman N, Seal S, Thompson D, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet*. 2007;39:165–167.
- [43] Casadei S, Norquist BM, Walsh T, et al. Contribution of inherited mutations in the BRCA2-interacting protein PALB2 to familial breast cancer. *Cancer Res*. 2011;71:2222–2229.
- [44] Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med*. 2014;371:497–506.
- [45] Cybulski C, Carrot-Zhang J, Kluzniak W, et al. Germline RECQL mutations are associated with breast cancer susceptibility. *Nat Genet*. 2015;47:643–646.
- [46] Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science (New York, NY)*. 2008;322:881–888.
- [47] Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*. 2007;447:1087–1093.
- [48] Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet*. 2007;39:870–874.
- [49] Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*. 2007;39:865–869.
- [50] Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*. 2008;40:703–706.

- [51] Ahmed S, Thomas G, Ghousaini M, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet.* 2009;41:585–590.
- [52] Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet.* 2009;41:324–328.
- [53] Thomas G, Jacobs KB, Kraft P, 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;41:579–584.
- [54] Turnbull C, Ahmed S, Morrison J, et al. Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet.* 2010;42:504–507.
- [55] Antoniou AC, Wang X, Fredericksen ZS, et al. 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;42:885–892.
- [56] Fletcher O, Johnson N, Orr N, et al. Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. *J Natl Cancer Inst.* 2011;103:425–435.
- [57] Haiman CA, Chen GK, Vachon CM, et al. A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. *Nat Genet.* 2011;43:1210–1214.
- [58] Ghousaini M, Fletcher O, Michailidou K, et al. Genome-wide association analysis identifies three new breast cancer susceptibility loci. *Nat Genet.* 2012;44:312–318.
- [59] Siddiq A, Couch FJ, Chen GK, et al. A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11. *Hum Mol Genet.* 2012;21:5373–5384.
- [60] Gaudet MM, Kuchenbaecker KB, Vijai J, et al. Identification of a BRCA2-specific modifier locus at 6p24 related to breast cancer risk. *PLoS Genet.* 2013;9:e1003173.
- [61] Couch FJ, Wang X, McGuffog L, et al. Genome-wide association study in BRCA1 mutation carriers identifies novel loci associated with breast and ovarian cancer risk. *PLoS Genet.* 2013;9:e1003212.
- [62] French JD, Ghousaini M, Edwards SL, et al. Functional variants at the 11q13 risk locus for breast cancer regulate cyclin D1 expression through long-range enhancers. *Am J Hum Genet.* 2013;92:489–503.
- [63] Garcia-Closas M, Couch FJ, Lindstrom S, et al. Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nat Genet.* 2013;45:392–398.
- [64] Bojesen SE, Pooley KA, Johnatty SE, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. *Nat Genet.* 2013;45:371–384.
- [65] Chen F, Chen GK, Stram DO, et al. A genome-wide association study of breast cancer in women of African ancestry. *Hum Genet.* 2013;132:39–48.
- [66] Long J, Cai Q, Sung H, et al. Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. *PLoS Genet.* 2012;8:e1002532.
- [67] Milne RL, Burwinkel B, Michailidou K, et al. Common non-synonymous SNPs associated with breast cancer susceptibility: findings from the Breast Cancer Association Consortium. *Hum Mol Genet.* 2014;23:6096–6111.
- [68] Cai Q, Zhang B, Sung H, et al. Genome-wide association analysis in East Asians identifies breast cancer susceptibility loci at 1q32.1, 5q14.3 and 15q26.1. *Nat Genet.* 2014;46:886–890.
- [69] Michailidou K, Beesley J, Lindstrom S, et al. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. *Nat Genet.* 2015;47:373–380.
- [70] Michailidou K, Lindstrom S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature.* 2017;551:92–94.
- [71] Kilpivaara O, Vahteristo P, Falck J, et al. CHEK2 variant I157T may be associated with increased breast cancer risk. *Int J Cancer.* 2004;111:543–547.
- [72] Bougeard G, Sesboue R, Baert-Desurmont S, et al. Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families. *J Med Genet.* 2008;45:535–538.
- [73] Struwing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med.* 1997;336:1401–1408.
- [74] King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science (New York, NY).* 2003;302:643–646.
- [75] Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer.* 2012;12:68–78.
- [76] Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet.* 1998;62:676–689.
- [77] Easton DF, Pharoah PDP, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med.* 2015;372:2243–2257.
- [78] Spurdle AB, Whitley PJ, Thompson B, et al. BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J Med Genet.* 2012;49:525–532.
- [79] Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet.* 2013;45:353–361.
- [80] van der Groep P, Bouter A, van der Zanden R, et al. Re: germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. *J Natl Cancer Inst.* 2004;96:712–713.
- [81] van der Groep P, van der Wall E, van Diest PJ. Pathology of hereditary breast cancer. *Cell Oncol.* 2011;34:71–88.
- [82] Mavaddat N, Barrowdale D, Andrulis IL, et al. 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 Biomark Prev.* 2012;21:134–147.
- [83] Callahan MJ, Crum CP, Medeiros F, et al. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. *JCO.* 2007;25:3985.
- [84] Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. *Breast Cancer Linkage Consortium. Lancet.* 1994;343:692–695.
- [85] Moran A, O'Hara C, Khan S, et al. Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. *Fam Cancer.* 2012;11:235–242.
- [86] Thompson D, Easton D. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. *Am J Hum Genet.* 2001;68:410–419.
- [87] Chompret A, Brugieres L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br J Cancer.* 2000;82:1932–1937.
- [88] Mai PL, Best AF, Peters JA, et al. Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. *Cancer.* 2016;122:3673–3681.
- [89] Hisada M, Garber JE, Fung CY, et al. Multiple primary cancers in families with Li-Fraumeni syndrome. *J Natl Cancer Inst.* 1998;90:606–611.
- [90] Hwang SJ, Lozano G, Amos CI, et al. Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. *Am J Hum Genet.* 2003;72:975–983.
- [91] Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat.* 2007;28:622–629.
- [92] Birch JM, Blair V, Kelsey AM, et al. Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. *Oncogene.* 1998;17:1061–1068.

- [93] Olivier M, Goldgar DE, Sodha N, et al. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. *Cancer Res.* 2003;63:6643–6650.
- [94] Nelen MR, Padberg GW, Peeters EA, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat Genet.* 1996;13:114–116.
- [95] Farooq A, Walker LJ, Bowling J, et al. Cowden syndrome. *Cancer Treatment Rev.* 2010;36:577–583.
- [96] Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. *J Med Genet.* 2013;50:255–263.
- [97] Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. *Clin Cancer Res.* 2012;18:400–407.
- [98] Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. *J Med Genet.* 1997;34:1007–1011.
- [99] Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clin Cancer Res.* 2006;12:3209–3215.
- [100] Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science.* 1991;251:1451–1455.
- [101] Pharoah PD, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology.* 2001;121:1348–1353.
- [102] Benusiglio PR, Malka D, Rouleau E, et al. *CDH1* germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. *J Med Genet.* 2013;50:486–489.
- [103] Petridis C, Shinomiya I, Kohut K, et al. Germline CDH1 mutations in bilateral lobular carcinoma in situ. *Br J Cancer.* 2014;110:1053–1057.
- [104] Xia B, Sheng Q, Nakanishi K, et al. Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell.* 2006;22:719–729.
- [105] Ramus SJ, Song H, Dicks E, et al. Germline mutations in the BRIP1, BARD1, PALB2, and NBN genes in women with ovarian cancer. *JNCI: J Natl Cancer Inst.* 2015;107. DOI:10.1093/jnci/djv214
- [106] Jones S, Hruban RH, Kamiyama M, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science (New York, NY).* 2009;324:217.
- [107] Matsuo S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science.* 1998;282:1893–1897.
- [108] Nevanlinna H, Bartek J. The CHEK2 gene and inherited breast cancer susceptibility. *Oncogene.* 2006;25:5912–5919.
- [109] Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. *BioMed Res Int.* 2013;2013:1.
- [110] Schmidt MK, Hogervorst F, van Hien R, et al. Age- and tumor subtype-specific breast cancer risk estimates for CHEK2*1100delC carriers. *JCO.* 2016;34:2750–2760.
- [111] Bogdanova N, Enßen-Dubrowskaja N, Feshchenko S, et al. Association of two mutations in the CHEK2 gene with breast cancer. *Int J Cancer.* 2005;116:263–266.
- [112] Cybulski C, Wokołorczyk D, Huzarski T, et al. A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland. *Breast Cancer Res Treat.* 2007;102:119–122.
- [113] Kriege M, Hollestelle A, Jager A, et al. Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy. *Br J Cancer.* 2014;111:1004–1013.
- [114] Weischer M, Nordestgaard BG, Pharoah P, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. *JCO.* 2012;30:4308–4316.
- [115] Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci USA.* 2011;108:18032–18037.
- [116] Cybulski C, Gorski B, Huzarski T, et al. CHEK2 is a multiorgan cancer susceptibility gene. *Am J Hum Genet.* 2004;75:1131–1135.
- [117] Xiang HP, Geng XP, Ge WW, et al. Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility. *Eur J Cancer (Oxford, England: 1990).* 2011;47:2546–2551.
- [118] Bogdanova N, Feshchenko S, Schurmann P, et al. Nijmegen Breakage Syndrome mutations and risk of breast cancer. *Int J Cancer.* 2008;122:802–806.
- [119] Zhang G, Zeng Y, Liu Z, et al. Significant association between Nijmegen breakage syndrome 1 657del5 polymorphism and breast cancer risk. *Tumor Biol.* 2013;34:2753–2757.
- [120] Steffen J, Nowakowska D, Niwińska A, et al. Germline mutations 657del5 of the NBS1 gene contribute significantly to the incidence of breast cancer in Central Poland. *Int J Cancer.* 2006;119:472–475.
- [121] Ahmed M, Rahman N. ATM and breast cancer susceptibility. *Oncogene.* 2006;25:5906–5911.
- [122] Uziel T, Lerenthal Y, Moyal L, et al. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* 2003;22:5612–5621.
- [123] Goldgar DE, Healey S, Dowty JG, et al. Rare variants in the ATM gene and risk of breast cancer. *Breast Cancer Res.* 2011;13:R73.
- [124] Grant RC, Selander I, Connor AA, et al. Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer. *Gastroenterology.* 2015;148:556–564.
- [125] Johannessen CM, Reczek EE, James MF, et al. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci USA.* 2005;102:8573–8578.
- [126] Uusitalo E, Leppavirta J, Koffert A, et al. Incidence and mortality of neurofibromatosis: a total population study in Finland. *J Invest Dermatol.* 2015;135:904–906.
- [127] Seminog OO, Goldacre MJ. Age-specific risk of breast cancer in women with neurofibromatosis type 1. *Br J Cancer.* 2015;112:1546–1548.
- [128] Madanik SA, Bergner A, Ye X, et al. Increased risk of breast cancer in women with NF1. *Am J Med Genet.* 2012;158a:3056–3060.
- [129] Coulet F, Fajac A, Colas C, et al. Germline RAD51C mutations in ovarian cancer susceptibility. *Clin Genet.* 2013;83:332–336.
- [130] Le Calvez-Kelm F, Oliver J, Damiola F, et al. RAD51 and breast cancer susceptibility: no evidence for rare variant association in the Breast Cancer Family Registry study. *PLoS One.* 2012;7:e52374.
- [131] Pelttari LM, Khan S, Vuorela M, et al. RAD51B in familial breast cancer. *PLoS One.* 2016;11(5):e0153788.
- [132] Park DJ, Lesueur F, Nguyen-Dumont T, et al. Rare mutations in XRCC2 increase the risk of breast cancer. *Am J Hum Genet.* 2012;90:734–739.
- [133] Rafnar T, Gudbjartsson DF, Sulem P, et al. Mutations in BRIP1 confer high risk of ovarian cancer. *Nat Genet.* 2011;43:1104–1107.
- [134] Seal S, Thompson D, Renwick A, et al. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet.* 2006;38:1239–1241.
- [135] Easton DF, Lesueur F, Decker B, et al. evidence that protein truncating variants in BRIP1 are associated with breast cancer risk: implications for gene panel testing. *J Med Genet.* 2016;53:298–309.
- [136] Thompson ER, Doyle MA, Ryland GL, et al. Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. *PLoS Genet.* 2012;8:e1002894.
- [137] Gracia-Aznarez FJ, Fernandez V, Pita G, et al. Whole exome sequencing suggests much of non-BRCA1/BRCA2 familial breast cancer is due to moderate and low penetrance susceptibility alleles. *PLoS One.* 2013;8:e55681.
- [138] Kiiski JI, Pelttari LM, Khan S, et al. Exome sequencing identifies FANCM as a susceptibility gene for triple-negative breast cancer. *Proc Natl Acad Sci USA.* 2014;111:15172–15177.

- [139] Damiola F, Pertesi M, Oliver J, et al. Rare key functional domain missense substitutions in MRE11A, RAD50, and NBN contribute to breast cancer susceptibility: results from a Breast Cancer Family Registry case-control mutation-screening study. *Breast Cancer Res: BCR*. 2014;16:R58.
- [140] Purrington KS, Slager S, Eccles D, et al. Genome-wide association study identifies 25 known breast cancer susceptibility loci as risk factors for triple-negative breast cancer. *Carcinogenesis*. 2014; 35:1012–1019.
- [141] Milne RL, Kuchenbaecker KB, Michailidou K, et al. Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat Genet*. 2017;49:1767–1778.
- [142] Palomaki GE. Is it time for BRCA1/2 mutation screening in the general adult population? Impact of population characteristics. *Genet Med*. 2015;17:24–26.
- [143] Margolin S, Werelius B, Fornander T, et al. BRCA1 mutations in a population-based study of breast cancer in Stockholm County. *Genet Test*. 2004;8:127–132.
- [144] Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst*. 1999;91:943–949.
- [145] Antoniou AC, Hardy R, Walker L, et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. *J Med Genet*. 2008; 45:425–431.
- [146] Thompson D, Easton D. The genetic epidemiology of breast cancer genes. *J Mammary Gland Biol Neoplasia*. 2004;9:221–236.
- [147] Stratton MR, Rahman N. The emerging landscape of breast cancer susceptibility. *Nat Genet*. 2008;40:17–22.
- [148] Antoniou AC, Pharoah PD, McMullan G, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. *Br J Cancer*. 2002;86:76–83.
- [149] Antoniou AC, Easton DF. Models of genetic susceptibility to breast cancer. *Oncogene*. 2006;25:25:5898–5905.
- [150] Antoniou AC, Spurdle AB, Sinilnikova OM, et al. Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Am J Hum Genet*. 2008;82:937–948.
- [151] Antoniou AC, Sinilnikova OM, McGuffog L, et al. Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Hum Mol Genet*. 2009;18: 4442–4456.
- [152] Antoniou AC, Kuchenbaecker KB, Soucy P, et al. Common variants at 12p11, 12q24, 9p21, 9q31.2 and in ZNF365 are associated with breast cancer risk for BRCA1 and/or BRCA2 mutation carriers. *Breast Cancer Res: BCR*. 2012;14. DOI:10.1186/bcr3121
- [153] Antoniou AC, Kartsonaki C, Sinilnikova OM, et al. Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Hum Mol Genet*. 2011;20:3304–3321.
- [154] Muranen TA, Greco D, Blomqvist C, et al. Genetic modifiers of CHEK2*1100delC associated breast cancer risk. *Genet Med*. 2017;19(5):599–603.
- [155] Turnbull C, Seal S, Renwick A, et al. Gene-gene interactions in breast cancer susceptibility. *Hum Mol Genet*. 2012;21:958–962.
- [156] Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*. 2003;72: 1117–1130.
- [157] CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. *Am J Hum Genet*. 2004;74:1175–1182.
- [158] Thompson D, Duedal S, Kirner J, et al. Cancer risks and mortality in heterozygous ATM mutation carriers. *J Natl Cancer Inst*. 2005; 97:813–822.
- [159] Peltomaki P. Update on Lynch syndrome genomics. *Fam Cancer*. 2016;15:385–393.
- [160] Harkness EF, Barrow E, Newton K, et al. Lynch syndrome caused by MLH1 mutations is associated with an increased risk of breast cancer: a cohort study. *J Med Genet*. 2015;52:553–556.
- [161] Murali R, Wiesner T, Scolyer RA. Tumours associated with BAP1 mutations. *Pathology*. 2013;45:116–126.
- [162] Horcasitas DJ, LaDuca H, Yussuf A, et al. Exploring a possible relationship of germline CDKN2A mutations with breast cancer in a multigene panel cohort. *JCO*. 2017;35:e23218.
- [163] von Wachenfeldt A, Lindblom A, Gronberg H, et al. A hypothesis-generating search for new genetic breast cancer syndromes – a national study in 803 Swedish families. *Hered Cancer Clin Pract*. 2007;5:17–24.
- [164] Wendt C, Lindblom A, Arver B, et al. Tumour spectrum in non-BRCA hereditary breast cancer families in Sweden. *Hered Cancer Clin Pract*. 2015;13:15.
- [165] Chang CQ, Yesupriya A, Rowell JL, et al. A systematic review of cancer GWAS and candidate gene meta-analyses reveals limited overlap but similar effect sizes. *Eur J Hum Genet*. 2014;22: 402–408.