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## Genetics of breast cancer: a topic in evolution

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A hereditary predisposition to breast cancer significantly influences screening and follow-up recommendations for high-risk women. However, in patients with a suggestive personal and/or family history, a specific predisposing gene is identified in <30% of cases. Up to 25% of hereditary cases are due to a mutation in one of the few identified rare, but highly penetrant genes (*BRCA1*, *BRCA2*, *PTEN*, *TP53*, *CDH1*, and *STK11*), which confer up to an 80% lifetime risk of breast cancer. An additional 2%–3% of cases are due to a mutation in a rare, moderate-penetrance gene (e.g. *CHEK2*, *BRIP1*, *ATM*, and *PALB2*), each associated with a twofold increase in risk. Prediction models suggest that there are unlikely to be additional yet to be identified high-penetrance genes. Investigation of common, low-penetrance alleles contributing to risk in a polygenic fashion has yielded a small number of suggestive single-nucleotide polymorphisms (SNPs), but the contributive risk of an individual SNP is quite small. Mutation testing is currently recommended for individual genes in the appropriate clinical setting where there is a high index of suspicion for a specific mutated gene or syndrome. Next-generation sequencing offers a new venue for risk assessment. At the present time, there are clear clinical guidelines for individuals with a mutation in a high-penetrance gene. Otherwise, standard models are used to predict an individual's lifetime risk by clinical and family history rather than genomic information.

**Key words:** breast cancer, family history, genetics, screening, multiplex gene panels, BRCA

## introduction

Breast cancer is the most common malignancy in women in Europe and the United States and second leading cause of cancer-related death. A recent publication estimated that there

were 464 000 cases of female breast cancer and 131 000 deaths in Europe in 2012 [1]. The American Cancer Society estimates that, in the United States, there were ~232 000 new breast cancer cases (of which 2000 were male breast cancer) and 40 000 deaths in 2013 [2]. There is no single definition of 'familial' breast cancer, but generally accepted criteria include: (i) at least three breast and/or ovarian cancer cases in a family;

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(ii) two breast cancer cases in close relatives, with at least one case diagnosed before age 50; (iii) two breast cancer cases in a family diagnosed before 40 years of age; (iv) any male breast cancer with a family history of ovarian cancer or early onset female breast cancer; (v) Ashkenazi Jewish ancestry with breast cancer, particularly triple-negative breast cancer diagnosed before age 60; or (vi) breast and ovarian cancer in the same patient [3, 4]. However, these criteria largely encompass only the phenotype of the Hereditary Breast/Ovarian Syndrome (HBOC; discussed in more detail below) and a predisposing gene is identified in <30% of cases with suggestive features [5]. The vast majority of familial cases are due to highly penetrant, but rare genes. In more recent years, additional rare, moderate-penetrance genes and common, low-penetrance alleles have also been identified. This paper will review the known genetic causes of breast cancer and discuss the issues associated with characterizing and understanding hereditary predispositions to breast cancer.

## high-penetrance genes

The first major gene associated with hereditary breast cancer was *BRCA1*, located on chromosome 17. This gene was identified in 1990 using linkage analysis in families with suggestive pedigrees [6]. In 1994, *BRCA2* was mapped to chromosome 13 [7]. A mutation in either *BRCA1* or *BRCA2* confers an increased risk of breast and other cancers. Large rearrangements and deletions in *BRCA1* or *BRCA2* can also alter the function of BRCA,

resulting in an identical clinical syndrome to that seen in carriers of mutations in these genes. The clinical syndrome seen in *BRCA* mutation carriers is referred to as the Hereditary Breast/Ovarian Cancer (HBOC) syndrome, though there are patients with this same clinical picture who are found to be negative for mutations in both *BRCA1* and *BRCA2*. Research in HBOC has focused on determining the associated risk of breast and other cancers, identifying specific clinical and histopathological features, and developing therapeutic and prevention strategies. Tumors due to mutations in *BRCA1* tend to be of the basal-like phenotype, have a high histologic grade, and do not commonly express the estrogen receptor (ER), progesterone receptor (PR), or Her2/neu, the so-called triple-negative tumor [8]. *BRCA2*-related tumors more closely resemble sporadic tumors [9].

*BRCA1* and *BRCA2* mutations are inherited in an autosomal dominant fashion, but act recessively on the cellular level as tumor suppressor genes involved in double-stranded DNA (dsDNA) break repair [5]. Female carriers of mutations in *BRCA1* or *BRCA2* have a lifetime risk of breast cancer of 50%–85% [10, 11]. Male carriers of *BRCA1* have an increased risk of breast cancer, though to a lesser degree than carriers of *BRCA2* who have an estimated 5%–10% lifetime risk [12]. Additional features of the syndromes are detailed in Table 1. Most notably, there is an increased risk of ovarian cancer, with an estimated lifetime risk of 10–40% for *BRCA1* carriers and 10%–20% for *BRCA2* carriers [10, 11, 13, 14]. Biallelic *BRCA2* mutations

**Table 1.** Breast cancer high-penetrance genes and their associated syndromes

Gene	Syndrome	Breast cancer incidence	Other associated cancers	Nonmalignant syndrome features
<i>BRCA1</i>	Hereditary	82% lifetime risk	Ovarian and fallopian tube cancer	
<i>BRCA2</i>	Breast/Ovarian Cancer Syndrome <sup>a</sup>		Prostate cancer Pancreas and biliary cancer Melanoma	
<i>PTEN</i>	PTEN Hamartoma Tumor Syndrome Cowden Syndrome	85% lifetime risk	Nonmedullary thyroid cancer Endometrial cancer GU tumors, especially renal cell carcinoma	Pathognomonic skin lesions Macrocephaly, benign breast and thyroid disease, uterine fibroids, Lhermitte–Duclos disease, fibromas, lipomas, intestinal hamartomas, mental retardation
<i>TP53</i>	Li-Fraumeni Syndrome	25% by age 74	Sarcoma Brain tumor Adrenocortical carcinoma Leukemia Lung bronchoalveolar cancer Multiple other cancers seen, but are more rare	
<i>CDH1</i>	Hereditary Diffuse Gastric Cancer	39% lifetime risk of lobular breast cancer	Gastric cancer, diffuse subtype Colorectal cancer	
<i>STK11</i>	Peutz-Jeghers Syndrome	32% by age 60	GI cancers (esophagus, stomach, small bowel, colon) Pancreatic cancer Sex-cord stromal tumors	GI hamartomatous polyposis Hyperpigmented macules Hyperestrogenism

<sup>a</sup>There are additional patients with this clinical phenotype, but without an identified mutation in either *BRCA1* or *BRCA2*.  
GI, gastrointestinal; GU, genitourinary.

manifest with the clinical picture of Fanconi anemia type D1 and greatly increase the risk of childhood cancers. Biallelic *BRCA1* mutations have very rarely been described [15], and are likely embryonic lethal in most cases [5].

Mutations and rearrangements or deletions in *BRCA1* and *BRCA2* are estimated to explain only 15% of familial breast cancers [5, 16]. There are subpopulations with higher frequencies due to founder mutations, most prominently the Ashkenazi Jewish population, in which three major mutations (*BRCA1*.185delAG, *BRCA1*.5382insC, and *BRCA2*.6174delT) alone account for ~10% of hereditary cases [10]. With sequencing and haplotype analysis, founder mutations and ethnic-group-specific mutations have also been demonstrated in other populations [17–20]. Additional rare, but highly penetrant genes include *PTEN* [21, 22], *TP53* [23–25], *CDH1* [26], and *STK11* [27, 28], each conferring a distinct clinical syndrome. These are described in Table 1. Collectively with *BRCA1* and *BRCA2*, it is estimated that the known high-penetrance genes account for no more than 25% of cases based on prior studies and mathematical modeling [16, 29].

It is crucial to recognize individuals with a hereditary cancer syndrome, as this greatly affects their clinical management. As detailed in the European Society for Medical Oncology (ESMO) [3] and National Comprehensive Cancer Network (NCCN) [4] guidelines, women with mutations in *BRCA1*, *BRCA2*, or one of the other high-penetrance genes should be counseled regarding breast awareness and breast self-exam starting at age 18. From age 25 (or 10 years before the youngest case in the family, whichever is earlier), clinical breast exam, and imaging with a combination of mammography and magnetic resonance imaging (MRI) is recommended annually. It is controversial which imaging modality is best used between ages 25 and 30, but annual MRI with consideration of annual mammogram is typically recommended [4, 30].

Risk-reducing salpingo-oophorectomy (RRSO) is recommended by age 35–40, or earlier if either child bearing is complete or there is indication based on the family history [31]. This substantially reduces the risk of ovarian cancer (though there is a residual risk of primary peritoneal cancer), and significantly reduces breast cancer risk if carried out before menopause [31, 32]. Prophylactic mastectomy may also be considered due to the high lifetime cancer risk and increased risk of secondary breast cancers, with discussion of a nipple-sparing approach [3, 33, 34]. For women who have not yet undergone RRSO, screening with pelvic ultrasound and serum CA-125 levels can be considered starting at age 30, though this has not clearly been shown to be beneficial [35, 36]. Limited data suggest that short-term hormone replacement therapy following RRSO may be considered for symptomatic benefit [37, 38].

Tamoxifen has been shown to reduce the risk of ER-positive breast cancer in women with an increased risk based on the Gail model, but has not been well studied in women with a known or suspected familial cancer syndrome. Limited clinical data suggest that tamoxifen may reduce risk of breast cancer in women with a *BRCA1* or *BRCA2* mutation who have not undergone prophylactic oophorectomy before menopause [39–41]. In a recently published observational study which followed 2464 *BRCA* mutation carriers [42], of whom 837 took tamoxifen following unilateral therapeutic mastectomy, there was a non-significant trend toward a lower risk of contralateral breast

cancer, with an adjusted hazard ratio (HR) of 0.58 [95% confidence interval (CI) 0.29–1.13] for *BRCA1* carriers and HR 0.48 (95% CI 0.22–1.05) for *BRCA2* carriers. Interestingly, in this analysis, the effect of tamoxifen did not vary by the estrogen status of the original breast cancer. A small sub-analysis of the Breast Cancer Prevention Trial that included *BRCA1* and *BRCA2* mutation carriers suggested a protective effect for tamoxifen only in women with *BRCA* mutations.

A recent meta-analysis suggested that ever use of oral contraceptives in *BRCA* mutation carriers or women with a strong family history of breast or ovarian cancer affected cancer risk similarly to the general population. Ovarian cancer risk was decreased [odds ratio (OR) 0.58, 95% CI 0.46–0.73], with no significant impact on breast cancer risk (OR 1.21, 95% CI 0.93–1.58) [43].

Medical treatment of hereditary breast cancer is generally dictated by histology, immunohistochemistry, and stage rather than knowledge of *BRCA* mutation status. Early clinical data suggest that *BRCA*-associated tumors are exquisitely sensitive to poly-[adenosine diphosphate (ADP)-ribose] polymerase (PARP) inhibitors, agents that inhibit the DNA damage repair mechanism PARP, but these are currently only available in the setting of clinical trials [44].

## moderate-penetrance genes

Linkage studies have failed to demonstrate additional reproducible loci for highly penetrant genes predisposing to breast cancer [16], although it should be noted that these analyses may not be powered to detect very rare high-penetrance genes. This has prompted new research directions for elucidating hereditary causes for breast cancer. A number of studies have focused on genes proposed to increase the risk of breast cancer based on their known cellular functions in families with pedigrees suggestive of a predisposition to breast cancer. Studies have identified a number of additional DNA repair genes that interact with *BRCA1*, *BRCA2*, and/or the *BRCA* pathways, and confer about a twofold increase in breast cancer risk, including *CHEK2* [45], *BRIP1* (*BACH1*) [46], *ATM* [47], and *PALB2* [48]. These genes and their mechanisms of action are listed in Table 2.

*CHEK2\*1100delC* is the most common mutation, seen in up to 1%–2% of the population; it is found in higher numbers in breast cancer patients, especially those with a family history or those who had negative *BRCA1* and *BRCA2* testing, where the prevalence may be as high as 5% [45]. *CHEK2* is a protein kinase involved in cell cycle regulation at G2 that is rapidly phosphorylated in response to DNA damage. Activated *CHEK2* stabilizes p53 and interacts with *BRCA1*. The *CHEK2\*1100delC* mutation confers about a twofold increase in female breast cancer and 10-fold increase in male breast cancer. Haplotype analysis has identified additional rare *CHEK2* mutations in the Ashkenazi Jewish population, suggestive of a founder effect [49]. There is no additional increase in risk for co-carriers of the *CHEK2* and *BRCA1* or *BRCA2* mutations, possibly due to an overlapping effect on DNA repair [45]. There is no known biallelic phenotype for *CHEK2*, again with the assumption that this is embryonic lethal [5].

*BRIP1* (*BACH1*) encodes a protein that interacts with the *BRCA1* C-Terminus (BRCT) domain of *BRCA1*. Mutations in *BRIP1* are thought to account for <1% of breast cancer cases.

**Table 2.** Breast cancer moderate-penetrance genes and associated breast cancer risks

Gene	Gene function	Breast cancer risk	Biallelic phenotype
CHEK2	Protein kinase involved in cell cycle regulation at G2. Rapidly phosphorylated in response to DNA damage. Activated CHEK2 stabilizes p53 and interacts with BRCA1	Female: RR 1.70, 95% CI 1.3–2.2 Male: RR 10.3, 95% CI 3.5–30.0	None known -presumed to be embryonic lethal
BRIP1 (BACH1)	Interacts with the BRCA1 C-Terminus (BRCT) domain of BRCA1	All women: RR 2.0, 95% CI 1.2–3.2 <50 years: RR 3.5, 95% CI 1.9–5.7	Fanconi anemia, type J -no significant increase in childhood cancers
ATM	Protein kinase involved in monitoring and repair of dsDNA and regulation of BRCA1 and CHEK2	RR 2.37, 95% CI 1.5–3.8	Ataxia-telangiectasia -autosomal recessive inheritance
PALB2	Associates with BRCA2. Involved in nuclear localization and stability	All women: RR 2.3, 95% CI 1.4–3.9 <50 years: RR 3.0, 95% CI 1.4–5.5	Fanconi anemia type N -higher incidence of childhood cancers

RR, relative risk; CI, confidence interval.

A mutation in *BRIP1* is associated with a relative risk (RR) of 2.0 in women with a strong family history of breast cancer, with a higher risk seen for early onset breast cancer. The majority of *BRIP1* mutations described to date are protein-truncating mutations. Biallelic *BRIP1* is associated with Fanconi anemia type J, without a significant increase in childhood cancers [46].

*ATM* is a protein kinase involved in monitoring and repair of dsDNA and regulation of BRCA1 and CHEK2. Biallelic *ATM* mutation causes the autosomal recessive disease ataxiatelangiectasia. The estimated prevalence of monoallelic *ATM* mutation is 1% [47]. In a recent meta-analysis, the RR of breast cancer associated with an *ATM* mutation was 2.3, with a higher risk seen for women under the age of 50 [50].

Mutations in these moderate-penetrance genes may be mediated by environmental factors, lessening their genetic impact. For example varying levels of selenium concentration have been suggested to impact the CHEK2- and ATM-dependent DNA repair pathways [51].

The encoded protein from *PALB2* associates with BRCA2 and is involved in nuclear localization and stability. It has an estimated incidence of 1%–2% and RR of 2.3 for all women and 3.0 in the subgroup of women under 50 years old [48, 52]. Biallelic *PALB2* mutation causes Fanconi anemia type N (clinically similar to type D1 seen with biallelic *BRCA2* mutations) and results in a higher incidence of childhood cancers. A higher incidence of *PALB2* mutations in male breast cancers has been described, though this likely only contributes to a minority of familial cases [53].

Additional genes involved in DNA damage repair, including *RAD51C* and genes in the MRN DNA repair pathway (*MRE11*, *RAD50*, *NBN* [*NBS1*]) have also been investigated. However, when high-risk families were screened, no mutations were clearly associated with increased cancer risk or with a specific clinical phenotype [54–58]. It is still possible that somatic mutations within tumors, or founder effects in unique populations, are present and contribute to cancer development and progression [49, 55, 59]. As an example, the *PALB2* c.1592delT founder

mutation occurs in ~1% of unselected Finnish breast cancer cases, but in one study was seen in 4.8% of cases with suggestive pedigrees and negative *BRCA* mutation testing [60].

Studies in the UK population have estimated that together, these moderately penetrant genes account for <3% of familial breast cancer, based on analyses of *BRCA* mutation-negative women with a personal or family history. These studies are often under-powered to comment on an earlier age of onset or other associated syndrome features [5]. Because these genes confer a lower lifetime risk of breast cancer than the highly penetrant genes described above, clinical management of women with mutations in moderate-penetrance genes, including screening and preventive interventions, is less clearly defined. Clinical management should incorporate risk assessment tools that incorporate both personal and family history and additional established breast cancer risk factors to determine risk. There are multiple options, including the Gail [61, 62], Claus [63], BRCAPRO [64], Tyrer-Cuzick [65], and BOADICEA [66] models, each of which evaluates slightly different demographic and history characteristics. For women with a calculated lifetime risk of breast cancer of at least 20% by virtue of a family history, annual breast MRI is recommended in addition to standard mammography. Of note, the Gail model only includes first-degree family history, and is therefore not recommended for justification of MRI screening [4, 67, 68]. Clinical breast examination every 6 months is currently recommended for all women with an increased lifetime risk of breast cancer [3, 4]. Both the Gail and Tyrer-Cuzick models have been used to establish eligibility for breast cancer chemoprevention studies [69, 70].

## low-penetrance alleles

As laboratory techniques have evolved and sequencing capabilities have advanced, genome-wide studies have been carried out to identify additional genetic variants that may contribute to breast cancer risk in a polygenic fashion. As it is still impractical to perform large whole-genome studies, instead sampling of

single-nucleotide polymorphisms (SNPs) distributed across the genome if often used to evaluate for genetic variability. SNPs are found in both genes and intergenic regions; variation in the latter of these can indicate variation in gene regulatory elements. These studies require thousands of cases and controls to have sufficient power to appreciate a change in risk, as individuals alleles may be relatively common and even found in a majority of the population [5]. An extremely stringent *P* value is required to minimize false positives from multiple testing, dividing  $P = 0.05$  by the number of SNPs tested, or as low as  $P = 5 \times 10^{-8}$  for an agnostic genome-wide search [16, 71].

A small number of polymorphisms in known breast cancer-associated genes have been associated with an increased risk of breast cancer. For example a Pro919Ser polymorphism in *BRIP1* has an odds ratio of 1.39 ( $P = 0.002$ ) in premenopausal women, but was not associated with an increased risk of breast cancer for the overall population [72]. Often, low-penetrance SNPs are located in noncoding regions of the genome (e.g. 2q35, 8q24), making it more difficult to identify an associated gene. The mechanism of increased cancer risk may be through activation of growth-promoting genes rather than inactivation of DNA repair, which is the most common mechanism seen for moderate- or high-penetrance genes. On average, each allele only mildly increases risk and is additive per allele rather than multiplicative, with odds ratios suggesting up to a 1.26-fold increase in risk for heterozygotes and 1.65-fold increase for homozygotes [5].

Thus far, the majority of studies have focused on one or a few variants at a time. However, a recent meta-analysis assessed the examined variants to date, excluding those in highly penetrant genes [73]. This analysis excluded the first report of a variant, small studies (<500 samples), and groups not deemed to be in Harvey–Weinberg equilibrium. Strong associations were seen in 10 variants across six genes [*ATM*, *CASP8* (cysteine-aspartic acid protease family with a role in apoptosis), *CHEK2*, *CTLA4* (encodes an inhibitory signal to T cells, affecting carcinogenesis via antitumor immunity), *NBN*, *TP53*] and a moderate association was noted in an additional four variants across four genes [*ATM*, *CYP19A1* (liver metabolic enzyme), *TERT* (enzyme that maintains chromosomal telomere ends), *XRCC3* (Rad51-related protein involved in DNA damage repair)]. Odds ratios >2 were seen for truncating mutations in *ATM* and *NBN* and for three rare variants in *CHEK2*. However, the remainder had a more minor calculated impact [73].

Evaluation for low-penetrance alleles is not currently part of standard clinical evaluation for breast cancer. Management of individuals found to carry these variants, as with moderate-penetrance genes, should be based on their estimated risk as calculated by the previously described validated risk assessment models.

## evaluation of suspected hereditary predisposition to breast cancer

Individuals with a family and personal history suspicious for a familial syndrome should be referred to a genetic counselor for a comprehensive evaluation. Testing for mutations in cancer-associated genes is individually based, and requires a high index of suspicion for a particular gene based on the clinical situation. In general, when a family history is suggestive, it is best to test

the individual with a cancer diagnosis, as this increases the probability of a positive test result. Standard clinical *BRCA1* and *BRCA2* testing has been carried out using PCR amplification and Sanger sequencing. For the Ashkenazi Jewish population, testing can be initially targeted to the three major founder mutations. In 2007, testing for large rearrangements was added for secondary analysis after research studies published that 6%–18% of individuals who are *BRCA* mutation negative by sequencing can be explained by large insertions and deletions in the *BRCA1* and *BRCA2* genes using multiplex ligation-dependent probe amplification technology [29, 74, 75]. If a mutation is identified, targeted testing can be done for other members of the family to assess risk. Possible outcomes of genetic testing are a true positive, a true negative (i.e. an individual in a family with a known mutation tests negative for that mutation), uninformative (i.e. a negative test in a family where a mutation has yet to be identified), or a variant of unknown significance (VUS). By definition, a VUS is a detected genetic change without a good description of any correlating clinical risk.

Traditionally, testing for patients with a suspected hereditary predisposition focused on evaluation for mutations in *BRCA1* and *BRCA2*, with possible additional testing based on specific family history. In the past several years, with the emergence of multiplex gene assays, there are additional options for evaluation. Before 2013, there was only one company in the United States with commercially available for *BRCA* testing. A Supreme Court decision in June 2013 laid the stage for other entities to be able to offer testing for *BRCA* mutations, and many of these now offer multigene panels that include *BRCA1* and *BRCA2*. Current testing options and the included genes are outlined in Table 3. Some of these assays are focused on genes associated with breast and/or ovarian cancer, while others contain a broader panel of cancer-associated genes, and lists of included genes are rapidly evolving. For example the Hereditary High-Risk Breast Cancer Panel (Baylor College of Medicine, Houston, TX) [80] focuses on the six high-penetrance genes as well as *PALB2*, while the BROCA assay (University of Washington, Seattle, WA, USA) [74, 76] has expanded to a panel of 49 genes, including a combination of high- and moderate-penetrance breast cancer-associated genes, genes associated with a high risk of colon cancer, and several promising low-penetrance genes. With next-generation sequencing, multiple genes can be tested for mutations at a fraction of the cost of individual gene sequencing [74]. Furthermore, this process may be useful in detecting mutation changes not identified by conventional sequencing, such as large rearrangements [86]. Multiplex gene testing may be especially helpful in patients with a rarer cause for their hereditary predisposition to cancer or women with a less obvious history, including those with fewer female relatives, paternal inheritance of the gene, and cases where few other relatives have inherited the predisposing gene [74]. Future research will also likely further define the role of modifier genes, and may more clearly identify the additive or synergistic effects of mutations or polymorphisms in multiple genes taken together.

As an example of the clinical application of panel testing, recently presented results from BROCA testing of 800 high-risk families who had negative commercial testing for *BRCA1* and *BRCA2*, revealed 206 (26%) with a positive BROCA assay. Of these, 80 (39%) had a previously undetected mutation in *BRCA1*

**Table 3.** Multiplex gene panels currently available for breast cancer risk analysis

Gene panel (Institution)	High-penetrance breast genes	Moderate-penetrance breast genes	Additional genes
BROCA [76] (University of Washington, Seattle, WA, USA)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>AKT1</i> , <i>APC</i> , <i>ATR</i> , <i>BABAM1</i> , <i>BAPI</i> , <i>BARD1</i> , <i>BMPR1A</i> , <i>CDK4</i> , <i>CDKN2A</i> , <i>CHEK1</i> , <i>CTNNA1</i> , <i>EPCAM</i> , <i>FAM175A</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>PIK3CA</i> , <i>PMS2</i> , <i>POLD1</i> , <i>POLE</i> , <i>PRSS1</i> , <i>RAD50</i> , <i>RAD51</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>RET</i> , <i>SDHB</i> , <i>SDHC</i> , <i>SDHD</i> , <i>SMAD4</i> , <i>TP53BP1</i> , <i>VHL</i> , <i>XRCC2</i>
ColoSeq [77] (University of Washington)	<i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>		<i>AKT1</i> , <i>APC</i> , <i>BMPR1A</i> , <i>EPCAM</i> , <i>GALNT12</i> , <i>GREM1</i> , <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>PIK3CA</i> , <i>POLD1</i> , <i>POLE</i> , <i>PMS2</i> , <i>SMAD4</i>
BreastNext [78] (Ambry Genetics, Aliso Viejo, CA, USA)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>BARD1</i> , <i>MRE11A</i> , <i>MUTYH</i> , <i>NBN</i> , <i>NF1</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i>
OvaNext [78] (Ambry Genetics)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>BARD1</i> , <i>EPCAM</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>NF1</i> , <i>PMS2</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i>
CancerNext [78] (Ambry Genetics)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>APC</i> , <i>BARD1</i> , <i>BMPR1A</i> , <i>CDK4</i> , <i>CDKN2A</i> , <i>EPCAM</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>PMS2</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>SMAD4</i>
Breast Cancer High-Risk Panel [79] (GeneDx, Gaithersburg, MD, USA)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>		
Breast/Ovarian Cancer Panel [79] (GeneDx)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>BARD1</i> , <i>BLM</i> , <i>EPCAM</i> , <i>FAM175A</i> , <i>FANCC</i> , <i>HOXB13</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH6</i> , <i>NBN</i> , <i>PMS2</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>XRCC2</i>
Comprehensive Cancer Panel [79] (GeneDx)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>APC</i> , <i>AXIN2</i> , <i>BARD1</i> , <i>BMPR1A</i> , <i>BLM</i> , <i>CDK4</i> , <i>CDKN2A</i> , <i>EPCAM</i> , <i>FAM175A</i> , <i>FANCC</i> , <i>HOXB13</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>PALLD</i> , <i>PMS2</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>SMAD4</i> , <i>VHL</i> , <i>XRCC2</i>
Hereditary High-Risk Breast Cancer Panel [80] (Baylor College of Medicine, Houston, TX, USA)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>PALB2</i>	
Hereditary Breast/Ovarian Cancer Panel [81] (Baylor College of Medicine)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>EPCAM</i> , <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>PMS1</i> , <i>PMS2</i> , <i>RAD51C</i> , <i>RAD51D</i>
Comprehensive Hereditary Cancer Panel [82] (Baylor College of Medicine)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>ALK</i> , <i>APC</i> , <i>BMPR1A</i> , <i>CDC73</i> , <i>CDKN1C</i> , <i>CDKN2A</i> , <i>EPCAM</i> , <i>FH</i> , <i>FLCN</i> , <i>GPC3</i> , <i>MAX</i> , <i>MEN1</i> , <i>MET</i> , <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i> , <i>MUTYH</i> , <i>NBN</i> , <i>NF1</i> , <i>NF2</i> , <i>PHOX2B</i> , <i>PMS1</i> , <i>PMS2</i> , <i>PRKAR1A</i> , <i>PTCH1</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>RET</i> , <i>SDHA2</i> , <i>SDHB</i> , <i>SDHC</i> , <i>SDHD</i> , <i>SMAD4</i> , <i>SUFU</i> , <i>TMEM127</i> , <i>VHL</i> , <i>WT1</i>
Breast Cancer Susceptibility [83] (City of Hope, Duarte, CA, USA)	<i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>CHEK2</i> , <i>PALB2</i>	
Hereditary Breast and Ovarian Cancer [84] (Sistemas Genómicos, Paterna, Spain)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>PALB2</i>	<i>ERCC4</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>XRCC2</i>
CAN02: Breast and Ovarian Cancer [85] (Center for Genomics and Transcriptomics, Tuebingen, Germany)	<i>BRCA1</i> , <i>BRCA2</i> , <i>CDH1</i> , <i>PTEN</i> , <i>STK11</i> , <i>TP53</i>	<i>ATM</i> , <i>BRIP1</i> , <i>CHEK2</i> , <i>PALB2</i>	<i>BARD1</i> , <i>EPCAM</i> , <i>FAM175A</i> , <i>FANCA</i> , <i>FANCC</i> , <i>FANCD2</i> , <i>FANCE</i> , <i>FANCF</i> , <i>FANCG</i> , <i>MEN1</i> , <i>MLH1</i> , <i>MRE11A</i> , <i>MSH2</i> , <i>MSH3</i> , <i>MSH6</i> , <i>NBN</i> , <i>PMS1</i> , <i>PMS2</i> , <i>PTCH1</i> , <i>RAD50</i> , <i>RAD51C</i> , <i>RAD51D</i> , <i>SLX4</i> , <i>UIMC1</i> , <i>XRCC2</i>

or *BRCA2* (either because large rearrangement testing had not been previously carried out or because one family member had negative testing, but a separate family member turned out to be a mutation carrier) and the remainder carried a mutation in a non-*BRCA* gene. This included high-penetrance genes (10 *TP53*, 1 *CDH1*, 1 *STK11*), moderate-penetrance genes (39 *CHEK2*, 28 *PALB2*, 15 *ATM*, 5 *BRIP1*), and poorly characterized genes (5 *RAD51D*, 4 *BARD1*, 3 *RAD51C*, 2 *ABRAXAS*, 2 *NBN*, 1 *XRCC2*) [87]. The benefit of detecting a high-penetrance non-*BRCA* gene mutation with an associated clinical syndrome is clear. However, the utility of recognizing a deleterious moderate- or low-penetrance gene mutation remains to be seen and may be more informative to future rather than current clinical practice. Thus, an argument could be made for using both a more restricted and a broader multigene panel.

As these panels evolve and become more complex, it is crucial to understand the context in which a particular patient underwent genetic testing, and which method was used. For example a woman who tested negative for *BRCA* mutations in 1998, when large rearrangement testing was not clinically available, could still potentially be found to be a *BRCA* mutation carrier if complete rearrangement testing was carried out [29]. Thus, the medical provider for a high-risk, but mutation-negative, breast cancer patient would need to recognize newer testing options that were not carried out as part of initial testing. Similarly, as multigene panels expand to more genes, simply reporting a 'negative panel test' would only be partially informative for which testing was done. There remains a burden on the clinician to be able to interpret not just positive and negative results, but also the context in which these results were obtained. At minimum, it would be helpful to always report the test result with the date of testing if the number of genes tested are too numerous to be included in a clinical summary.

With more detailed genetic analysis, and with the availability of multiplex assays, an increased amount of indeterminate information is often obtained. Next-generation sequencing testing will additionally require careful analysis and interpretation of VUS [88]. There are multiple *in silico* models aiming to postulate the functional significance of these variants [89], but current recommendations are to still treat these mutations as VUS until they are classified as deleterious.

Additionally, as costs for genomic assays have decreased, the number of commercially available assays billed as personal genomic testing (PGT) has increased substantially, but our ability to interpret the results of these assays remains limited. A number of tests are marketed directly to consumers, thus making it difficult for treating physicians to counsel patients regarding the value of testing. A major concern with this new avenue of medical risk assessment is that patients and physicians often feel under-informed regarding the interpretation of results. In a survey of over 10 000 physicians, 98% felt that PGT results may influence drug therapy, but only 10% believed they were adequately informed how to interpret the results [90]. In a survey of people who elected PGT testing, 10% discussed their results with the company genetic counselor and only 27% chose to share results with their physician, increasing risk that the test would be resulted without adequate counseling and test interpretation [91]. Limited data suggest that, in the appropriate clinical setting, PGT can be effective in modulating clinical behavior [90].

## Conclusion

In conclusion, despite decades of medical research, <30% of cases with a suggestive personal and/or family history of hereditary breast cancer have an identified causative gene mutation. The vast majority of these cases are due to a mutation in one of the highly penetrant breast cancer genes (*BRCA1*, *BRCA2*, *PTEN*, *TP53*, *CDH1*, and *STK11*) and there are current guidelines that provide concrete direction for the management of these patients. A minority of cases are due to mutations in moderate-penetrance genes (*CHEK2*, *ATM*, *BRIP1*, and *PALB2*). A small number of low-penetrance alleles have been identified using advanced genetic testing methods. While these may contribute to risk in a polygenic fashion, this is likely to be relevant to a minority of cases and their identification should not be considered routine practice. Mutation testing currently requires a high index of suspicion for a specific contributing etiology, but next-generation sequencing may improve the identification of such genes and the clinical management of these cases.

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## Disclosure

The authors have declared no conflicts of interest.

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