Biomarkers for Early Detection of Familial Breast Cancer

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ABSTRACT
Breast cancer (BC) is classified as sporadic, familial, or hereditary. In familial BC, an unusual high number of members in a family are affected by breast, ovarian, or a related cancer. Family history is crucial in determining an individual’s BC susceptibility. A person’s risk of developing BC increases with an increasing number of affected family members. Only 5%–10% of all BC appears to have strong inheritance. Of these, 4%–5% is caused by genes with high penetrance and transmitted in an autosomal dominant manner. Testing for well-characterized mutations in blood based on a clinical suspicion of familial cancer is a well-known approach for genetic testing in cancer susceptibility. This review summarizes known genetic biomarkers,
which may be used for early diagnosis of familial BC. Genes that serve as biomarkers for hereditary BC can be classified according to penetrance. BRCA1 and BRCA2, two BC-associated genes with high penetrance, account for about 5% of all BCs, and mutation rates of these genes are variable across populations. Other BC susceptibility genes that have been described as having high to moderate penetrance are CHEK2, PTEN, TP53, ATM, STK11/LKB1, CDH1, NBS1, RAD50, BRIP1, and PALB2. A further 20 low-penetrance BC risk-modifying alleles have been identified to date. These genes all likely play a role in the development and progression in the wide spectrum of observed hereditary BCs.

5.1 Introduction

5.1.1 What is Familial Breast Cancer?

BC is classified as sporadic, familial, or hereditary. Sporadic BC accounts for 70%–75% of cases and is thought to be due to nonhereditary causes. Hereditary or familial terms are used to express BC caused by inheritable germline mutations in germ cells. According to National Institute for Health and Care Excellence (NICE) clinical guidelines, if there are an unusual high number of members in a family affected by breast, ovarian, or a related cancer, this is typically called familial or hereditary BC. Development of BC depends on the nature of the family history; the number of relatives who have developed breast, ovarian, or a related cancer; age at which relatives developed BC; and the age of the person (NICE clinical guideline 2013).

5.1.2 Why Is It Important?

BC remains the leading cause of cancer-related death in women despite a substantial decrease in BC mortality over the past two decades. This decrease in mortality is due, in part, to the development and use of adjuvant therapies as well as better and more widespread early screening programs (Peto et al. 2000; EBCTCG 2005). In addition to the pain suffered by cancer patients and their families, cancer places a significant economic burden on society as a whole. The estimated annual worldwide incidence is 1,383,000 (Lynch et al. 2012). Since cancer incidence is usually higher among older people, an aging population will increase the overall cost of cancer treatment in the future. Better diagnostic markers are needed to reduce the various personal and societal costs associated with this disease (Jemal et al. 2005; Lai et al. 2012).

There are many risk factors for BC development, including reproductive history, obesity, and hormonal factors (Turkoz et al. 2012). However, family history remains the most important associated factor, with risk increasing as the number of affected family members increases. Hereditary BC affects several family members across multiple generations. Most of these cancers are caused by mutations in the highly penetrant BC-associated genes. About 5%–10% of all BC has a strong inheritance while 25%–40% of BCs occurring before the age of 35 is hereditary in nature (Lux et al. 2006). Three to eight percent of all BC is caused by mutations in BRCA1 and BRCA2, which are probably the best and most widely known BC-related genes. In familial BC cases,
they account for up to 40% of all cases (Lux et al. 2006). Mutation rates of these highly penetrant genes vary from population to population (Kurian et al., 2010).

Other genes that have been described as high to moderately penetrant BC susceptibility genes are CHEK2, PTEN, TP53, ATM, STK11/LKB1, CDH1, NBS1, RAD50, BRIP1, and PALB2 (Lalloo and Evans 2012). All of these genes play a role in the observed spectrum of hereditary BCs. Large number of genes with moderate to low penetrance may be responsible for BC cases, and a combination of these may contribute to a significant proportion of familial BC (Renwick et al. 2006; Rahman et al. 2007; Stratton and Rahman 2008; Mealiffe et al. 2010; Turnbull et al. 2010; Shuen and Foulkes 2011). Despite recent discoveries of new genetic susceptibility factors, there still remains a substantial portion of familial hereditary BC without any relationship to known BC susceptibility genes.

5.1.3 Early Detection of Familial BC

Easily detectable mutations in genes and proteins from patient blood samples are ideal diagnostic markers. Identifying biomarkers is immensely important to help medical doctors identify individuals who may be susceptible to certain cancer types. Biomarkers are also useful for distinguishing between different stages of cancer and providing more accurate prognoses. These biomarkers should be cancer-type specific and detectable in a wide range of specimens containing cancer-derived materials, including body fluids, tissues, and cell lines.

Mutations in several genes are associated with certain types of hereditary BC. As a gold standard, Sanger sequencing has been used to detect genetic-based mutations in blood. However, Sanger-based mutation detection faces difficulties of screening lots of samples at one time. Recent development of next-generation sequencing (NGS) provides the analysis of many genes at a time using commercial test panels. Testing for well-characterized mutations based on a clinical suspicion of familial cancer is through the approach of genetic testing for cancer susceptibility.

Early detection of cancer results in longer patient survival and reduces the amount of required treatment. There has been a considerable investment in the early detection of cancer over the past few decades. Screening programs for the early diagnosis of cancers that can detect asymptomatic malignancies or premalignant lesions are helpful for both patients and doctors. Early detection is one way to limit the human suffering associated with cancer as well as reduce the burden it places on society. Detection of cancer is related to how easily noticeable a tumor is. According to data from the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute, in the United States, a 5-year survival rate for patients diagnosed with skin cancer approaches 95%, and for BC patients, this figure ranges from 75% to 90%, while patients diagnosed with lung or pancreatic cancer have dramatically lower 5-year survival rates of 15% and 6%, respectively (http://seer.cancer.gov/statfacts/html/melan.html). This is due, in part, to the ease with which the former cancer types can be observed as compared with the latter types.

Detailed information regarding diagnosis of hereditary BC is available from several sources based in the United States, including American Society of Clinical Oncology (www.asco.org), the US Preventive Services Task Force (www.ahrq.gov/clinic/uspstfix.thm), and the National Comprehensive Cancer Network (www.nccn.org). Also available is a web-based tool offered by the US Department of Health and Human Services, which can be found at http://www.hhs.gov/familyhistory (Gage et al. 2012).
5.1.4 Genetics of BC

BRCA1 and BRCA2 were the first genes to be linked to hereditary BC. They were discovered in the 1990s by linkage analysis and positional cloning using pedigrees with familial BC in successive generations (Miki et al. 1994; Wooster et al. 1995). BRCA1 and BRCA2 mutation carriers not only are susceptible to breast and ovarian cancers (Narod et al. 1991; Rigakos and Razis 2012) but also have an increased risk for developing melanoma (Cruz et al. 2011; Iscovich et al. 2002), as well as cancers of the fallopian tube (Kauff and Barakat 2007; Crum et al. 2012), colon and stomach (Brose et al. 2002), prostate (Kirchhoff et al. 2004; Moran et al. 2012), and pancreas (Hahn et al. 2003; Bartsch et al. 2012).

Although disease-related mutations in BRCA1 and BRCA2 have high penetrance and in individuals with mutations in these genes, the risk of BC increases by 10- to 20-fold; they are rare in the population with a frequency between 0.2% and 1% (Kurian 2010; Paradiso and Formenti 2011). The lifetime risk associated with mutations in various genes associated with BC varies from study to study depending on a variety of factors, including population studied, methods of sample selection, and methods of analysis. However, it is generally accepted that in women, BRCA1 mutations confer an overall lifetime risk of developing BC of about 65% and 40% for developing ovarian cancer. BRCA2 mutations confer a risk of about 45% for BC in females and 6% in males and an approximately 11% risk of developing ovarian cancer.

Apart from the BRCA genes, there are a number of other genes that collectively account for some portion of non-BRCA1-/non-BRCA2-associated familial BC. Furthermore, it is possible that other low to moderate penetrance genes may account for the heterogeneity in tumor types associated with clearly BRCA1- or BRCA2-associated BCs, the so-called polygenic model (Shuen and Foulkes 2011). Furthermore, rare copy number variants (CNVs) that disrupt p53 and estrogen receptor pathways may contribute to hereditary BC risk in cases that are negative for mutations in genes known to confer increased BC risk (Pylkas et al. 2012). BC has been reported to be part of the Lynch syndrome in some families. Lynch syndrome is characterized by a predisposition to colon cancer, and the mismatch repair proteins MLH1, MSH2, and MSH6 are known to play a role in this condition. It is thought that the absence of MLH1 and MSH2 proteins may play a role in these BC cases. MSH6 may also be involved, but only one case has been reported thus far (Shanley et al. 2009; van der Groep et al. 2011).

5.2 High-Risk Genes

5.2.1 BRCA1

The early 1990s saw a number of papers published in which regions of chromosome 17q were identified as being associated with breast and/or ovarian cancer (Hall et al. 1990). In 1994, Miki et al. reported the positional cloning of a candidate BRCA1 gene and identification of possible disease-associated mutations in patients with suspected 17q-associated breast or ovarian cancer (Miki et al. 1994). The BRCA1 tumor suppressor is estimated to be responsible for approximately 8% of familial BC (Lalloo and Evans 2012). In the NCBI PubMed database, a search for BRCA1 and BC retrieves over 7000 records, highlighting the significance of this gene in our understanding of this highly heterogeneous disease. Pathogenic mutation of BRCA1 is observed in about 1 in every
1000 individuals (Lalloo et al. 2003), and such mutations increase the risk of developing BC in both women and men (Tai et al. 2007).

Based on a 2004 review of the literature by Liede et al., it appears that BRCA1 mutations may account for approximately 4% of all male BC. Higher incidence has been reported in some studies, but these are typically based on cohorts that include patients who sought genetic counseling or were from families with known BRCA mutations (Liede et al. 2004). BRCA1 mutations may also contribute somewhat to the risk of prostate cancer in men under 70 years of age; however, the relationship is not very strong. Evidence for the association of BRCA2 mutations in both male breast and prostate cancers is much stronger (Liede et al. 2004).

A recently published multistage genome-wide association study (GWAS) of BRCA1 carriers attempted to identify risk-modifying loci. Several loci that appear to significantly modify risk were found. The authors estimated that 5% of BRCA1 carriers with the lowest risk have a lifetime risk of developing BC of 28%–50% and that the 5% of carriers with the highest risk have a lifetime risk of 81%–100% (Couch et al. 2013).

A retrospective study of radiation exposure from routine diagnostic tests carried out before the age of 30 was associated with higher incidence of BC in carriers of BRCA1 and BRCA2 mutations as compared to the general population (Pijpe et al. 2012). This result has very significant implications for the use of various diagnostic tests performed in families with known BRCA mutations.

BRCA1 is a large gene containing a total of 24 exons. It plays a key role in several cellular functions including DNA repair, chromatin remodeling, and transcriptional regulation (Poehlmann and Roessner 2010). Its role in DNA repair is primarily as part of homologous recombination (HR) repair that is required for the resolution of replication-associated double-strand breaks. In the absence of competent HR repair, the error-prone nonhomologous end joining (NHEJ) pathway is utilized thus compromising genome stability (Roy et al. 2012). Therapies that are aimed at multiple DNA repair pathways can sensitize otherwise therapy-resistant cancers (Stachelek et al. 2010; Yap et al. 2011). Targeted therapies capable of exploiting the HR repair deficiency in BRCA-deficient tumors have had some degree of success. An example is the use of poly ADP-ribose polymerase (PARP) inhibitors in combination with, for example, a drug-like temozolomide, to create a sort of synthetic lethality. PARP is involved in the repair of DNA via the base excision repair (BER) pathway. In the absence of PARP, lesions such as 3-methyladenine and 7-methylguanine that are normally repaired by BER are left unrepaired (Figure 5.1a). Subsequently, during replication, these normally nonleterious lesions may be transformed into toxic lesions via replication fork stalling and collapse and the formation of single- and double-strand breaks (Figure 5.1b) (Trivedi et al. 2005, 2008; Fishel et al. 2007). Thus, PARP inhibition and BRCA deficiency combined with treatment using a DNA-damaging agent such as temozolomide lead to an overwhelming degree of damage that even cancer cells are incapable of tolerating (Yap et al. 2011).

### 5.2.1.1 Clinical Features of BRCA1

The breast tumors that develop in BRCA1 carriers differ from those that develop in nonfamilial BCs. BRCA1 BCs are generally characterized by an increased frequency of pushing margins, high degree of nuclear pleomorphism, and mitotic frequency (Lakhani et al. 2005; de la Cruz et al. 2012; Lalloo and Evans 2012). The diagnosis of BC in BRCA1 carriers typically occurs at a younger age. Prognosis of these BCs is quite variable, with a range of disease-free survival similar to that seen in sporadic BCs. BRCA1 tumors are typically hormone receptor negative and aggressive in nature (Paradiso and Formenti 2011).
FIGURE 5.1
(a) Main steps involved in short-patch base excision repair. DNA lesions are recognized by lesion-specific DNA glycosylases. The base is excised, and an endonuclease cuts the sugar–phosphate backbone. DNA polymerase modifies DNA ends and fills gaps with appropriate base, and backbone is then religated, restoring DNA to its original state. In this model, PARP1 protects single-strand breaks during repair process. (b) Glycosylases recognize and remove damaged DNA base. In the absence of proteins required to carry out repair steps downstream of damage recognition (i.e., PARP inhibition), abasic sites and single-strand breaks accumulate. In tumor cells deficient in homologous recombination repair, exhibiting the so-called BRCAness, cells are overwhelmed with damage and cannot survive.
**BRCA1** and triple-negative BCs frequently exhibit a similar genomic profile as assayed by array comparative genomic hybridization (aCGH) and share a similar presence of lymph node metastasis (Vollebergh et al. 2011). According to results from a comprehensive international epidemiological collaboration, the proportion of estrogen-receptor-negative and triple-negative tumors in BRCA1 carriers appears to decrease with age at diagnosis (Mavaddat et al. 2012). The same study found that medullary tumors are more common in BRCA1 carriers (Mavaddat et al. 2012).

BRCA1 cancers have a similar immunohistological profile to sporadic basal carcinoma and are positive for CK5/6/CK14 (Lakhani et al. 2005; Laloo and Evans 2012). BRCA1 tumors typically express basal markers like basal keratins, P-cadherin, and epidermal growth factor receptor (Palacios et al. 2008).

### 5.2.2 BRCA2 (FANCD1)

Like BRCA1, BRCA2 was identified in the early to mid-1990s amidst the search for BC susceptibility genes. After a second susceptibility locus was identified at 13q12, researchers began probing that region to find the associated gene. In 1995, Wooster et al. discovered BRCA2 by positional cloning and identified germ line mutations in several BC families (Collins et al. 1995; Wooster et al. 1995). Also like BRCA1, BRCA2 is associated with an increased risk of developing both breast and ovarian cancers. BRCA2 mutations are found in 10% of families with a significant history of BC and confer an overall lifetime risk of about 40%–85% for women and 6% for men (Lalloo and Evans 2012). Note that the risk of developing BC in males with BRCA2 mutations is much higher than in males with BRCA1 mutations. BRCA2 mutations have also been associated with an increased risk for developing other types of cancers such as pancreatic (Huang et al. 2013; Leung and Saif 2013; Mocci et al. 2013) and prostate (Sundararajan et al. 2011; Castro and Eeles 2012; Sandhu et al. 2013).

BRCA2 is a large gene with a total of 24 exons. Like BRCA1, it is involved in DNA repair by HR (Roy et al. 2012). Thus, cancers with BRCA2 deficiency may also respond to therapies that target multiple DNA repair pathways (Clark et al. 2012). Interestingly, in a study that examined biopsies from patients whose cancers initially responded to PARP inhibition treatment and subsequently became resistant, secondary mutations in BRCA2 were identified that actually resulted in restoration of full-length BRCA2 protein (Barber et al. 2013).

BRCA2 has been shown to interact with a number of other proteins that have been implicated in the pathogenesis of BC, including RAD51, TP53, and PALB2 (Clark et al. 2012; Roy et al. 2012). Biallelic mutations in BRCA2 are responsible for a small percentage of children with Fanconi anemia (FA), a disease characterized by progressive marrow failure, congenital anomalies, and predisposition to malignancy (Myers et al. 2012). BRCA2-associated FA typically has an earlier age of onset, and patients are more likely to have leukemia and solid tumors (Myers et al. 2012).

#### 5.2.2.1 Clinical Features of BRCA2

Unlike BRCA1, BCs with BRCA2 mutations do not appear to have a specific characteristic pathology. However, they do tend to exhibit features more common to sporadic BCs than those associated with BRCA1 (Roy et al. 2012). BRCA2 tumors are more likely to be estrogen and progesterone receptor positive (Palacios et al. 2008). Both BRCA1 and BRCA2 mutation carriers are more likely than non-BRCA mutation carriers to have high expression of HIF1α (a key regulator of the response to hypoxia) in ductal carcinoma in situ (DCIS), a precancerous lesion known to progress to invasive BC (van der Groep et al. 2013).
Mitotic rates in BRCA2 tumors range from being lower than those observed in sporadic tumors to higher (Lalloo and Evans 2012). There is an apparent negative correlation between age at diagnosis of BRCA2 carriers and proportion of estrogen-receptor-negative/triple-negative status. The same study found that lobular tumors are more common in BRCA2 carriers (Mavaddat et al. 2012). The same study found that lobular tumors are more common in BRCA2 carriers (Mavaddat et al. 2012). A specific BRCA2 mutation, 999del5, was found to be associated with high-grade, rapidly proliferating tumors in a study that compared 40 families harboring this mutation against an age-matched control group (Agnarsson et al. 1998). Thirty-four out of 40 BRCA2 999del5 mutation-harboring patients had ductal carcinoma. The BRCA2 999del5 tumors also had less tubule formation, more nuclear pleomorphism, and higher mitotic rates as compared with controls (Agnarsson et al. 1998).

5.2.3 TP53

In 1979, DeLeo et al. identified a protein with a molecular weight of 53 kDa in mouse cells transformed by various methods (DeLeo et al. 1979). In 1981, a protein of the same size was identified in both SV40-transformed human cell lines and cells derived from spontaneous tumors (Crawford et al. 1981). The gene that encodes the protein identified by these groups came to be known as TP53 (or p53). TP53 is a tumor suppressor and is the most frequently altered gene in human tumors. Somatic mutations in the TP53 gene commonly occur in solid tumors, and at least 250 TP53 germ line mutations have been identified to date (Ognjanovic et al. 2012). TP53 mutations have been reported to be particularly common in triple-negative and BRCA1-associated BCs (Palacios et al. 2008; Walerych et al. 2012). Also, the spectrum of TP53 mutations in BRCA-associated tumors is unique as compared to sporadic tumors, with differences in mutation distribution and base changes (Palacios et al. 2008).

Germ line mutations in TP53 usually result in childhood cancers or early onset of tumors in adults. Autosomal dominant germ line TP53 mutations are responsible for the majority (~80%) of families affected by Li–Fraumeni syndrome (LFS) (Ognjanovic et al. 2012) and is also associated with some cases (15%–25%) of Li–Fraumeni-like syndrome (LFL) (Ognjanovic et al. 2012). LFS is a cancer syndrome that was first described by Li and Fraumeni in 1969 (Varley 2003). It is characterized by early-onset cancer with a characteristic tumor spectrum including sarcomas, brain and breast tumors, and childhood adrenocortical carcinoma (Varley 2003). LFS families are highly radiosensitive, and about 50% of LFS individuals will develop some kind of cancer before the age of 30. This is in contrast to the 1% chance that individuals in the general population have of developing cancer by 30 years of age (Sorrell et al. 2013). Frebourg et al. began studying in earnest the link between germ line TP53 mutations, LFS, and BC in 1991 (Frebourg et al. 1991). It is now known that about 30% of female mutation carriers will develop BC by 30 years of age. Furthermore, the development of secondary cancers later in life is also a high likelihood in LFS individuals (Sorrell et al. 2013). According to Macciari et al. (2012), most DCIS and invasive ductal carcinomas occurring in LFS patients who have TP53 germ line mutations are hormone receptor positive and/or HER2 positive. Thus, hormone receptor status as well as the radiosensitivity associated with LFS should be taken into account when determining possible treatments. Although LFS is responsible for less than 0.1% of BC, there is an 18- to 60-fold increased risk of BC for individuals under 45 years of age as compared to the general population and therefore is an important BC syndrome to be aware of (Lalloo and Evans 2012).
TP53 has 11 exons with a core DNA-binding domain encoded by exons 4–8 (Lalloo and Evans 2012). TP53 is often referred to as the guardian of the genome (Carson and Lois 1995; Sigal and Rotter 2000) and is involved in many cellular processes via transcription regulation and signaling networks including DNA damage response, regulation of cell cycle arrest, apoptosis, and senescence (Lalloo et al. 2003; Rubbi and Milner 2003). TP53 has been reported to interact with as many as 100 different proteins. Because TP53 is so essential to many cellular processes involved in maintaining genome integrity, it is hardly surprising that mutations in this gene are so common in human cancers. For this reason, it and the pathways that it is involved in are common targets for cancer therapy. As recently reviewed by Morandell and Yaffe (2012), therapeutic modalities that target tumor suppressors/oncogenes together with DNA damage pathways (much like the aforementioned PARP inhibitor plus DNA-damaging drug strategy) have been successfully applied both in vitro and in vivo, including treatment of human cancers.

5.2.4 PTEN

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene containing nine exons and is located at chromosome 10q23.3. PTEN encodes the protein phosphatidylinositol phosphate phosphatase protein. PTEN was identified in 1997 after deletions in the region of chromosome 10q23-24 were identified in greater than 90% of glioblastoma multiformes (Steck et al. 1997). Some 1900 different alterations, mutations, and deletions in PTEN have been identified thus far. Allelic or complete deletion of PTEN occurs in breast and prostate cancers. PTEN’s tumor suppressor activity is probably related to its function as a lipid phosphatase, which is involved in the regulation of the PI3K–AKT–mTOR pathway. It may also be related to its function as a protein phosphatase that is necessary for carrying out its activity in cell cycle arrest and inhibition of invasion in vitro (Hollander et al. 2011). PTEN localization to the nucleus may be necessary for repair of double-strand breaks mediated by RAD51, another gene that has been implicated in some types of BCs (Hollander et al. 2011). PTEN also regulates the tumor suppressor activity of APC and CDH1 in the nucleus (Song et al. 2011).

Germ line mutations result in rare conditions that are collectively referred to as PTEN hamartoma tumor syndromes (PTHS). Two PTHS-inherited syndromes are Cowden syndrome (CS) and Bannayan–Riley–Ruvalcaba syndrome (BRRS). PTEN mutations have also been identified in brain and prostate cancers (Li et al. 1997). PTEN expression is downregulated in BRCA1-associated cancers exhibiting a basal-like phenotype (Campeau et al. 2008).

CS is an autosomal dominant disorder whose diagnosis is difficult (Eng 2010). While PTEN deletion in mice can cause Cowden-like phenotypes and spontaneous tumorgenesis in some tissues, it is likely that the combined disruption of several pathways is responsible for the development of most PTEN-associated cancers (Hollander et al. 2011). Eighty percent of CS patients harbor germ line mutations in PTEN and typically develop cancers of the breast, thyroid, and endometrium. Somatic PTEN mutations are also commonly seen in sporadic tumors of these same types (Hollander et al. 2011). In women with CS, the lifetime risk of developing BC is 25%–50%. In sporadic BCs, although only 5% has PTEN mutations, 40% exhibits loss of PTEN expression as determined by immunoreactivity (Hollander et al. 2011).

It is unclear whether BRRS is a distinct clinical condition or simply an early-onset type of CS (Gustafson et al. 2007; Lachlan et al. 2007). Lachlan et al. (2007) suggest that BRRS and CS are a single clinical entity, mostly differing in the age of onset, much like the tumor suppressor disorder neurofibromatosis type 1. On the other hand, Gustafson et al.
maintain that the two are clinically distinct syndromes as BRRS is not associated with an increased incidence of malignancy. However, they also recommend that any individuals identified as having PTEN mutations should follow cancer surveillance recommendations for CS (Gustafson et al. 2007).

5.2.5 STK11

Serine/threonine kinase 11 (STK11), also known as LKB1, is located on chromosome 19p13.3 and encodes a serine/threonine kinase that regulates cell polarity and functions as a tumor suppressor. As a kinase upstream of AMPK, it also is involved in the maintenance of metabolic homeostasis. Its loss may result in hyperactivation of the mTOR pathway in HER2-positive BC (Andrade-Vieira et al. 2013). In addition to BC, STK11 has been studied in connection with non–small cell lung cancer (Shackelford et al. 2013) and colorectal cancer (Ngeow et al. 2013).

Germ line mutations in STK11 occur in the autosomal dominant condition known as Peutz–Jeghers syndrome (PJS), which is characterized by perioral pigmentation and intestinal hamartomatous polyposis. PJS is also associated with a predisposition to various types of cancers including those of the breast, cervix, testicles, and ovarian sex cord (Hemminki 1999). Patients with this syndrome have an approximate risk of 30% of developing BC by the age of 60 (Lim et al. 2004; Hearle et al. 2006). In a study of cancer incidence in 240 PJS patients with STK11 mutations, Lim et al. identified mutations in exon 3 of STK11 that appear to be associated with the greatest risk of developing cancer. However, these mutations may only be related to gastrointestinal cancers and not those of the breast. Missense and truncating mutations in regions other than exon 3 all had similar degrees of cancer risk (Lim et al. 2004). Due to the function of STK11 in the mTOR pathway, it is possible that rapamycin could be used to treat patients with PJS-associated cancers (Campeau et al. 2008).

5.2.6 E-Cadherin (CDH1)

Cadherin 1 (CDH1) or E-cadherin is located at chromosome 16q22.1 and encodes the protein E-cadherin, a calcium-dependent cell adhesion glycoprotein that is important for cell–cell adhesion (Becker et al. 1994). Approximately 30%–40% of familial diffuse gastric cancer (FDGC), an autosomal dominant syndrome, results from mutations in CDH1, and affected women are predisposed to lobular BC (Keller et al. 1999; Schrader et al. 2008). Patients with FDGC have an approximate risk of BC of between 39% and 52% (Schrader et al. 2011). Schrader et al. (2011) reported that in women with early-onset or familial lobular BC but without a family history of diffuse gastric cancer, mutations in CDH1 are rare.

5.3 Moderate Penetrance Genes

5.3.1 ATM

Ataxia telangiectasia mutated (ATM) is located on chromosome 11q22.3 and encodes a checkpoint kinase involved in DNA damage signaling. Biallelic mutations cause a rare, autosomal recessive disorder called ataxia telangiectasia (AT) (Savitsky et al. 1995), which is characterized by a host of somatic disorders. Heterozygous mutations of ATM do not
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result in the AT phenotype; however, they increase an individual’s sensitivity to radiation and raise the risk of BC by about 2.5-fold (Thompson et al. 2005; Renwick et al. 2006; Laloo and Evans 2012).

The localization of p53 to the centrosome during mitosis is ATM dependent. Prodosmo et al. hypothesized that this feature could be used to distinguish between healthy A-T heterozygotes and A-T homozygotes. Indeed, by determining the percentage of mitotic lymphoblasts or PBMCs with p53 centrosomal localization, they were able to reliably, quickly, and inexpensively distinguish between these different genotypes. Healthy individuals had >75% p53 centrosomal localization, while A-T heterozygotes had 40%–56% and A-T homozygotes had <30%. In a preliminary study, their test confirmed that ATM is a BC susceptibility gene (Prodosmo et al. 2013).

A recently published study of the in vivo therapeutic potential of ATM siRNA was investigated. A gene-specific ATM siRNA was delivered using a well-characterized porous silicon-based multistage vector (MSV) delivery system (MSV/ATM). Treatment included biweekly administration of siRNA to BALB/c mice. Their immune response was monitored through serum measurement of cytokines, chemokines, and colony-stimulating factors. The drug delivery system/siRNA did not trigger any acute immune response in the mice, and other parameters such as body weight, blood chemistry, and major organ histology did not result in any significant changes even after 4 weeks of treatment. Furthermore, successful knockdown of ATM was achieved, and the growth of MDA-MB-231 orthotopic tumors in nude mice was inhibited. This report opens up future possibilities for modulation of DNA repair pathways for therapeutics in humans (Xu et al. 2013).

5.3.2 CHEK2 (CHK2)

Checkpoint kinase 2 (CHEK2) is located on chromosome 22q12.1 and encodes a cell cycle checkpoint kinase involved in the DNA damage response (Matsuoka et al. 1998; Wu et al. 2001). It is located upstream of p53 and downstream of ATM in the DNA damage repair signaling network. CHEK2 is a tumor suppressor gene, and somatic mutations as well as aberrant expression have been identified in a number of malignancies, including osteosarcoma and ovarian cancer (Miller et al. 2002), lymphomas (Hoglund et al. 2011; Ferrao et al. 2012), colon cancer (Meijers-Heijboer et al. 2003; Stawinska et al. 2008), and glioblastoma multiforme (Ferrao et al. 2012). In a study of uterine serous carcinoma (USC) employing targeted capture and massively parallel sequencing to assess the status of 30 different tumor suppressor genes, 5% of the 151 patients examined had germ line mutations in BRCA1, CHEK2, or p53. The authors thus concluded that a small number of USC cases appear to be associated with hereditary breast and ovarian cancer (Pennington et al. 2013).

It was previously thought that mutations in CHEK2 might account for a small percentage of LFS cases in which p53 mutations were not present (Bell et al. 1999; Lee et al. 2001; Varley 2003). However, after more thorough investigations, the case for this hypothesis was not able to be convincingly made (Tavor et al. 2001; Meijers-Heijboer et al. 2002; Stawinska et al. 2008; Hoglund et al. 2011).

Two germ line mutations, in particular, 1100delC and I157T, have been the focus of much research (Nevanlinna and Bartek 2006). The CHEK2*1100delC mutation is a truncating mutation that results in reduced protein kinase activity. The mutation frequency was calculated to be 1.1% in healthy European and North American populations and 5.1% in a group of 718 BRCA-negative BC families (Meijers-Heijboer et al. 2002). CHEK2 mutations are observed more often in patients with wild-type BRCA1 and BRCA2 than in patients who have mutations in these genes (Turnbull et al. 2012). When the CHEK2*1100delC
mutation does occur in BRCA mutation carriers, it does not result in any increased risk (Meijers-Heijboer et al. 2002). Based on a meta-analysis of 26,000 cases and 27,000 controls taken from unselected, early-onset, and familial BC studies, Weischer et al. (2008) concluded that the CHEK2*1100delC mutation results in a 37% risk of BC by the age of 70 in heterozygous carriers. According to a recent publication (Adank et al. 2011), women from families with homozygous CHEK2* 1100delC mutations have about a sixfold higher risk of developing BC. Based on these results, it may be recommended that patients in BC families be screened for CHEK2 in addition to BRCA1 and BRCA2. Kabacik et al. (2011) reported a potentially clinically useful test for assessing ATM/CHEK2/p53 pathway activity.

CHEK2 c.507delT is a loss-of-function mutation identified in a screen of CHEK2 mutations in families with known BC predispositions (Manoukian et al. 2011).

5.3.3 BRIP1/BACH1 (FANCJ)

The BRIP1 gene encodes a protein that is a binding partner of BRCA1 and has been investigated as a BC predisposing gene. As with BRCA2 biallelic mutations, such mutations in BRIP1/BACH1 also result in FA. In a study published by Tang et al., haplotype blocks constructed with 48 tag SNPs (tSNPs) were used to investigate the haplotypes of eight BC susceptibility genes (TP53, PTEN, CHEK2, ATM, NBS1, RAD50, BRIP1, and PALB2) in 734 female patients and 672 age-matched controls. Haplotypes within NBS1, PTEN, and BRIP1 were associated with increased sporadic BC risk. However, in this study, only haplotypes within NBS1 were found to be associated with increased familial BC risk (Tang et al. 2013).

In a study of high-risk individuals of Ashkenazi Jewish ethnic background, it was found that BRIP1 mutations were only marginally associated with BC risk (Catucci et al. 2012). It is thought that mutations in BRIP1 may also contribute to an increased risk for ovarian cancer (Rafnar et al. 2011).

5.3.4 PALB2 (FANCN)

The PALB2 gene encodes a protein that interacts with BRCA2 during HR and double-strand break repair. Heterozygous mutations in PALB2 result in increased risk for BC while homozygous mutations, as with BRIP1 and BRCA2, result in FA. It is unclear why these FA genes confer an increased risk for BC while other FA genes do not. It may be that they are involved in or associate with proteins involved in DNA repair by HR (Campeau et al. 2008). PALB2 mutations are found in about 1%–4% of families negative for BRCA1 or BRCA2 mutations (Poumpouridou and Kroupis 2012). Mutations in PALB2 have also been associated with ovarian (Teo et al. 2013a; Tischkowitz et al. 2013) and pancreatic cancers (Axilbund and Wiley 2012; Solomon et al. 2012).

A rare mutation, PALB2 c.3113G>A (p.Trp1038*), which results in a protein-truncating mutation and is associated with a risk of BC similar in magnitude to that of BRCA2, has been identified in Australian patients (Teo et al. 2013b). In another study originating from the same lab, a previously unreported PALB2 mutation, PALB2c.1947_1948insA, was identified in a mutation screen of 748 Australasian multiple-case BC families. In this study, it was also determined that the c.3113G>A mutation occurs at a frequency of 1.5% in this population. Most of the cancers arising in carriers of any one of the 22 PALB2 mutations identified in this study were high-grade invasive ductal carcinomas (Teo et al. 2013b). Fibroblast cell lines derived from patients with heterozygous PALB2 c.229delT mutation displayed
evidence of chromosomal rearrangements and altered centromere distribution. However, due to extremely low sample size, the relevance of this result is not clear (Wark et al. 2013). Pathogenic truncating \textit{PALB2} mutations previously identified in family studies were identified in mutations screening of lymphocyte DNA in a population-based study and were estimated to confer a 5.3-fold increase in risk of developing BC. They also reported that, at least based on their obtained results, rare missense mutations in \textit{PALB2} do not confer a strong BC risk (Tischkowitz et al. 2012).

\textit{PALB2} 1592delT is a mutation that has been observed in BC families negative for mutations in \textit{BRCA1} and \textit{BRCA2}. Most of these tumors were triple negative and ductal and exhibited a high-grade phenotype. They were also mostly CK5/6, CK14, and CK17 negative and showed high expression of Ki67 and low expression of cyclin D1 as compared to other familial cases and sporadic BCs (Heikkinen et al. 2009; van der Groep et al. 2011).

\section*{5.4 Low-Penetrance BC Genes}

There have been many studies in recent years in which BC predisposing SNPs have been identified. Many of these have been extremely large collaborative efforts which, along with advances in sequencing technology, have allowed scientists to move closer to understanding the complicated genetics of hereditary and sporadic BC. Table 5.1 contains a list of SNPs identified as having some degree of risk for BC in \textit{BRCA1} and \textit{BRCA2} mutation carriers. The table includes some SNPs that confer a decrease in disease risk. Also included is the gene \textit{BARD1}, as a recent report suggests that this gene has no association with BC risk and thus resources should be funneled away from its study and directed toward more promising genes.

According to initial reports, \textit{RAD51C} was identified as a high-risk gene for both breast and ovarian cancers. Later, research showed this gene to be predominantly associated with increased risk of ovarian cancer while the risk of BC is not clearly elevated (Lalloo and Evans 2012).

\section*{5.5 Conclusion and Future Directions}

People with familial BC need a special approach because of differences in the BC risk. Family history is the most important factor for determining BC risk, and this risk grows with an increasing number of affected relatives with BC. The first high-risk BC genes were discovered in the 1990s, and since then, there has been intense research to find more BC susceptibility genes. BC is highly heterogeneous disease, complicating our understanding, diagnosis, and treatment of it. Recent advances in technology have greatly improved our ability to probe the genome for subtle changes. Although many of the genes that have been associated with hereditary BC are known to play a role in DNA repair, particularly the repair of double-strand breaks by HR repair, it is likely that most of hereditary and sporadic BC is caused by multiple genes from more than one pathway. The massive population-based GWASs are increasing our understanding of this deadly disease with each passing day. In time, our understanding of the roles played by many of the genes described in this chapter may provide better diagnostic and therapeutic tools for those
TABLE 5.1
Low-Penetrance SNPs and Gene Mutations that Modify Hereditary BC Risk in BRCA1 and BRCA2 Mutation Carriers

<table>
<thead>
<tr>
<th>(Nearby) Gene</th>
<th>Locus</th>
<th>SNP/Variant</th>
<th>Hazard Ratio (HR)/Assoc. with BRCA1 or BRCA2 Carriers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>6p24</td>
<td>rs9348512</td>
<td>0.85/BRCA2 reduced risk</td>
<td>Gaudet et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>12q24</td>
<td>rs1290211</td>
<td>0.92/BRCA2</td>
<td>Antoniou et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>9q31.2</td>
<td>rs865686</td>
<td>BRCA2</td>
<td>Antoniou et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>9q31</td>
<td>rs865686</td>
<td>0.99/BRCA2</td>
<td>Antoniou et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>11q13</td>
<td>rs614367</td>
<td>1.08/BRCA2</td>
<td>Antoniou et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>5p12</td>
<td>rs10941679</td>
<td>1.07/BRCA2</td>
<td>Antoniou et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>20q13</td>
<td>rs311498</td>
<td>0.95/BRCA2</td>
<td>Couch et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>12q24</td>
<td>rs13281615</td>
<td>1.03/BRCA2</td>
<td>Antoniou et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>8q24</td>
<td>rs4733664</td>
<td>1.10/BRCA2</td>
<td>Gaudet et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>19p13.1</td>
<td>rs15987042</td>
<td>1.17/BRCA1</td>
<td>Couch et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>2q35</td>
<td>rs16917302</td>
<td>1.14/BRCA1</td>
<td>Antoniou et al. (2009)</td>
<td></td>
</tr>
<tr>
<td>1q32</td>
<td>rs2290854</td>
<td>1.14/BRCA1</td>
<td>Couch et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>6q22.33</td>
<td>rs2180341</td>
<td>Weak effect—may decrease risk BRCA1</td>
<td>Kirchhoff et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>CDKN2A/B</td>
<td>9p21</td>
<td>rs1011970</td>
<td>1.03/BRCA2</td>
<td>Antoniou et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10965163</td>
<td>0.94/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td>ZNF365</td>
<td>10q21.2</td>
<td>rs10995190?</td>
<td>0.84/BRCA2</td>
<td>Antoniou et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs16917302</td>
<td>0.88/BRCA2</td>
<td>Couch et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs17231319</td>
<td>1.09/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td>MERIT40</td>
<td>19p13</td>
<td>rs81700</td>
<td>0.98/BRCA2</td>
<td>Couch et al. (2012)</td>
</tr>
<tr>
<td>ZMIZ1</td>
<td>10q22</td>
<td>rs704010</td>
<td>1.01/BRCA2</td>
<td>Antoniou et al. (2012)</td>
</tr>
<tr>
<td>STXB4, COXII</td>
<td>17q23</td>
<td>rs6504950</td>
<td>1.04/BRCA2</td>
<td>Antoniou et al. (2010)</td>
</tr>
<tr>
<td>PTHLH</td>
<td>12p11</td>
<td>rs27633</td>
<td>1.14/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td>MAP3K1</td>
<td>5q11</td>
<td>rs889312 rs16886113</td>
<td>1.10/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.24/BRCA2</td>
<td></td>
</tr>
<tr>
<td>PTHLH</td>
<td>12p11</td>
<td>rs10771399</td>
<td>0.87/BRCA1</td>
<td>Antoniou et al. (2012)</td>
</tr>
<tr>
<td>FGFR2</td>
<td>10q26</td>
<td>rs2981582</td>
<td>1.26/BRCA2</td>
<td>Gaudet et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2420946</td>
<td>0.96/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td>TOX3</td>
<td>16q12</td>
<td>rs3803662</td>
<td>1.20/BRCA2</td>
<td>Gaudet et al. (2010)</td>
</tr>
<tr>
<td>NOTCH2</td>
<td>1p11</td>
<td>rs11249433</td>
<td>1.05/BRCA2</td>
<td>Antoniou et al. (2011)</td>
</tr>
<tr>
<td>BARD1</td>
<td></td>
<td>Cys557Ser and haplotypes</td>
<td>Not assoc. with increased risk</td>
<td>Spurdle et al. (2011)</td>
</tr>
<tr>
<td>XRCC1</td>
<td></td>
<td>pArg280His-rs25489</td>
<td>BRCA2d</td>
<td>Osorio et al. (2011)</td>
</tr>
<tr>
<td>MORF4L1</td>
<td></td>
<td>rs7164529 rs10519219</td>
<td>BRCA2—weak assoc.</td>
<td>Martrat et al. (2011)</td>
</tr>
<tr>
<td>ESR1</td>
<td>6q25.1</td>
<td>rs2046210</td>
<td>1.17/BRCA1</td>
<td>Antoniou et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs9397435</td>
<td>1.14/BRCA2</td>
<td>Antoniou et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2253407</td>
<td>0.92/BRCA2</td>
<td>Gaudet et al. (2013)</td>
</tr>
<tr>
<td>RAD51L1</td>
<td>1p11.2</td>
<td>rs16942</td>
<td>1.14/BRCA2</td>
<td>Antoniou et al. (2011)</td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
<td>Decreased risk</td>
<td>Cox et al. (2011)</td>
</tr>
</tbody>
</table>

(Continued)
Biomarkers for Early Detection of Familial Breast Cancer

NGS is a featured method for screening hereditary BC. It allows for the analysis of multiple genes at a time using commercial panels. In future, more biomarkers would be revealed to enlighten hereditary BC, and NGS panels would be more accessible and comprehensive to detect mutations from blood.

References


**TABLE 5.1 (CONTINUED)**

Low-Penetrance SNPs and Gene Mutations that Modify Hereditary BC Risk in BRCA1 and BRCA2 Mutation Carriers

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>SMAD3</td>
<td>15q22</td>
<td>rs71660081</td>
<td>1.25/BRCA2</td>
<td>Walker et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3825977</td>
<td>1.20/BRCA2</td>
<td></td>
</tr>
<tr>
<td>RHAMMM</td>
<td>HMMR</td>
<td></td>
<td>1.09/BRCA1</td>
<td>Maxwell et al. (2011)</td>
</tr>
<tr>
<td>LSP1</td>
<td></td>
<td>rs3817198</td>
<td>1.16/BRCA2</td>
<td>Antoniou et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.11/BRCA2</td>
<td>Antoniou et al. (2010)</td>
</tr>
<tr>
<td>IRS1</td>
<td></td>
<td>rs1801278</td>
<td>1.86/class II BRCA1 mutations</td>
<td>Ding et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Gly972Arg)</td>
<td>0.86/class I BRCA1 mutations</td>
<td></td>
</tr>
<tr>
<td>PHB</td>
<td></td>
<td>rs6917 (1630 C &gt; T)</td>
<td>Rare homozygote genotype that may modify risk/BRCA1</td>
<td>Jakubowska et al. (2012)</td>
</tr>
</tbody>
</table>

a Risk restricted to mutations proven or predicted to lead to the absence of protein expression; associated primarily with estrogen receptor (ER)-negative BC in BRCA1 carriers (HR 0.82).
b Associated mostly with ER-negative BC in both BRCA1 and BRCA2.
c As it is a BRCA1/2 interactor, BARD1 has been commonly studied as possible modifier of BC risk.
d In a small series of samples (701 BRCA1 carriers and 576 BRCA2 carriers), in BRCA2 mutations carriers, rare homozygotes for this mutation had increased risk (HR = 22.3). However, when repeated with larger sample size (4480 BRCA1 and 3016 BRCA2), no evidence of an association was found.
e In BRCA1 mutation carriers who also carry this rare SNP on the WT BRCA1 allele, the risk of BC is decreased; SNP may cause nuclear proteins to have higher affinity for BRCA1. Low-penetrance variants, identified through genome-wide association studies (GWASs), are common but associated with smaller increases in risk (Turnbull et al. 2012). Some of these act as modifiers of BRCA1 and BRCA2. A total of 19 validated SNPs have been reported thus far (Turnbull et al., 2010).
f Appears to act multiplicatively in BRCA2 mutation carriers.


Biomarkers for Early Detection of Familial Breast Cancer


Biomarkers for Early Detection of Familial Breast Cancer


