Breast Cancer Genetics

8

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Abbreviations

Endo BX	Endometrial biopsy
HBOC	Hereditary breast and ovarian cancer
HDGC	Hereditary diffuse gastric cancer
MMG	Mammography
MRI	Magnetic resonance imaging
RRSO	Risk-reducing salpingo-oophorectomy
TVUS	Transvaginal ultrasound

8.1 Introduction

Breast cancer is the most common form of cancer and the second most common cause of cancer death among women in the developed world. It has been estimated that women in the USA have a 12% lifetime risk of developing breast cancer beginning in their 20s, with a risk of developing cancer in the next 10 years for a woman in her 30s of approximately one in 250, and 1 in 50 by age 50 [1]. In the modern era, the goal is to identify women at increased risk to try to prevent their breast cancers. Currently, it is well known that individual risk for breast cancer is increased in individuals carrying a mutation in a predisposing gene and in others with a number of affected relatives with early age of disease onset in whom no specific mutation has been identified [2]. Approximately 5–10% of the total breast cancer cases follow a Mendelian inheritance pattern (autosomal dominant) and are characterized as hereditary. An additional 15–20% of breast cancer cases are named familial, referring to women who have two or more first- or second-degree relatives with the disease, without an identified gene. Among hereditary breast cancer cases, at least 30% are caused by germline mutations in the high-penetrance genes *BRCA1* and *BRCA2* [2], and the risk associated with less prevalent and more moderately penetrant genes is the subject of intense research effort.

Knowledge of somatic genetics and genomics has increasingly broad implications in oncology, not only in the identification of new treatments such as trastuzumab for HER2-positive breast cancer [3] but also as the basis for assays evaluating recurrence risk and treatment guidance, such as Oncotype DX, MammaPrint, and PAM50 [4, 5]. The germline or heritable genome provides important implications for the identification of high-risk individuals, ultimately for the development of effective cancer prevention strategies across the tumor types for which the genes confer increased cancer risk. However, there have been implications for therapeutic interventions as well. Prominent examples of the latter include the data for cis- and carboplatins in *BRCA1/2*-associated breast and ovarian cancers [6, 7] and PARP inhibitors in *BRCA1/2*-associated breast, ovarian, pancreatic, and prostate cancers. For this chapter, the ultimate goal of germline information is to identify individuals and families not yet affected, but at high risk of developing tumors who might be interested in preventive interventions that might effectively reduce their cancer mortality, at least for those cancers for which they have greatest risk.

The evolution of next-generation sequencing technologies has enabled parallel simultaneous testing of multiple genes beyond *BRCA1/2*, leading to concurrent analysis of breast cancer predisposition genes with a range of associated cancer risks, including high- and intermediate-/moderate-penetrance genes. The efficiency of next-generation sequencing has also increased the speed of the analysis, thereby reducing turnaround time, and has significantly reduced the costs. The effect of the widespread introduction of NGS technologies, therefore, has been to increase access to more comprehensive genetic analyses. However, access to this technology has also brought new challenges: the identification of the ideal candidates for utilization of panels, the

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appropriate management of patients with mutations in genes whose penetrance still is not clear, the increase in the number of variants of uncertain significance (VUS) found, and the incidental finding of mutations in genes in families that do not have a clear phenotype of that syndrome.

8.2 Genetic Testing

Patients with breast cancer should be offered genetic testing, according to consensus guidelines. The guidelines may differ in some specifics depending on the country, but most would concur that individuals with breast cancer should be tested if they are diagnosed at a young age (< age 50 is a frequent criterion), when they present with triple-negative histology, or with ovarian cancer, and recently castrate-resistant prostate cancer or pancreatic cancer. Individuals without cancer are often eligible for genetic testing based on family cancer history that includes the above tumors in various configurations.

The ESMO guidelines comprise widely accepted clinical criteria for referral for genetic evaluation of unaffected individuals with family histories as follows: three or more breast and/or ovarian cancer cases, at least one <50 years, two breast cancer cases <40 years, male breast cancer and ovarian cancer or early-onset female breast cancer, Ashkenazi Jewish individuals with breast cancer of <60 years, young onset bilateral breast cancer, and breast and ovarian cancer in the same patient. In some countries, the criterion for testing is based on an a priori 10–20% probability of finding a mutation based on predictive models such as BRCAPRO, BOADICEA, or Manchester score [8]. However, others believe these criteria are too strict [9].

Much of the early data came from studies of *BRCA1* and *BRCA2*, and still the most substantial and stable data come from the study of individuals with mutations in these genes. They were the first identified, and data come from large cohorts, including the CIMBA consortium, which is custodian for tens of thousands of *BRCA1/2* mutation carriers that have been systematically studied for more than 10 years [10]. For these patients the most well-established genes to be evaluated are *BRCA1/2*, especially because those are the most common genes involved in breast cancer susceptibility and also because those are the ones for which we have the best data regarding penetrance and management.

At this time, however, the availability of multigene panel testing has raised new issues regarding eligibility for gene testing beyond BRCA and new challenges about interpretation and management of the results. According to the National Comprehensive Cancer Network (NCCN) in the USA, patients who have a personal and family history suggestive of a specific syndrome may be best evaluated by a target gene analysis. For those whose history can be explained by more than one gene—which is the majority of patients—evaluation by panel can be more efficient and/or cost-effective. For those patients with *BRCA1/2*-negative tests and with a strong family history, panels can be a good option in increasing the chance of finding a mutation in another predisposing gene by about 4% [11].

In this chapter we will first present the most important genes related to breast cancer risk, detailing their prevalence, associations with different cancers, and any pathologic characterizations and/or molecular features of those cancers. We will then discuss the clinical management of individuals carrying significant alterations in each gene as regards surveillance, risk-reducing surgery, and other available treatment regimes.

8.3 High-Penetrance Genes

8.3.1 *BRCA1* and *BRCA2*: Hereditary Breast and Ovarian Cancer (HBOC) Syndrome

The first gene associated with hereditary breast cancer is *BRCA1*, located on chromosome 17q. This gene was identified in 1990 using linkage analysis in families with suggestive pedigrees [12]. In 1994, *BRCA2* was mapped to chromosome 13q, and together they became the most important and studied genes related to hereditary breast and ovarian cancers [13].

Female carriers of pathogenic variants (mutations) in *BRCA1* or *BRCA2* have a lifetime risk of breast cancer of 50–85% [14–16]. In addition, there is a substantially increased risk of ovarian cancer, with an estimated lifetime risk of 20–60% for *BRCA1* carriers and 10–20% for *BRCA2* carriers [14, 17]. There are other tumors associated with mutations in BRCA2 in particular, and cases of melanoma, prostate, and pancreatic cancer [18] should be taken into account when considering family history.

When considering histopathological features, it is well established that *BRCA1*-related breast tumors, as a group, differ from non-*BRCA1* tumors in terms of histological phenotype. Malignant primary breast tumors of *BRCA1* mutation carriers are more likely to be high grade with medullary subtype features, including greatly increased mitotic count, pushing margins, lymphocytic infiltrate, trabecular growth pattern, and necrosis. Most importantly, about 70% do not express estrogen or progesterone receptor or HER2 (triple-negative breast cancer—TNBC) [19], but perhaps 20% are positive for ER and PR, and the remaining 5–10% are HER2 positive [20, 21]. This distribution has led to recognition that a significant subset of TNBC will occur in women who carry a germline *BRCA1* mutation, even in the absence of family cancer history, and has made TNBC in women younger than age 60 at diagnosis, a criterion for BRCA testing.

The majority of *BRCA2*-associated tumors are invasive ductal, high-grade, estrogen and progesterone receptor positive and negative for HER2. They are less likely than controls to express the basal cytokeratin CK5 or to overexpress HER2/neu protein. In fact *BRCA2* tumors are predominantly high-grade invasive ductal carcinomas of no special type, and they demonstrate a luminal phenotype despite their high histologic grade [20, 22, 23].

Among hereditary breast cancer cases, at least 30% are attributed to germline mutations in the high-penetration genes *BRCA1* and *BRCA2*, but these numbers can vary across different populations due to founder effects [2, 24].

Evidence shows that, in addition to the presence of a mutation on *BRCA1/2*, other factors such as environment, lifestyle factors, mutation locations, and the presence of some SNPs might be important to precisely estimate the quantitative cancer risks associated with specific BRCA mutations in carriers [25] and may affect the clinical management of these patients in the future. Direct evidence for genetic modifiers of risk has been provided through studies that investigated the effects of common breast and ovarian cancer susceptibility variants on cancer risk for *BRCA1* and *BRCA2* mutation carriers, identified through genome-wide association studies (GWAS) or candidate gene studies in the general population [26–30]. The GWAS data required independent validation but could provide helpful stratification of risk to assist women with the planning of risk-reducing measures, childbirth, and other aspects of life. Another important issue, addressed in a large analysis of genotype/phenotype data published by Rebbeck et al. on behalf of the CIMBA consortium, is that breast and ovarian cancer risks vary with the precise location of the mutation in *BRCA1* or *BRCA2*. The clustering of mutations in the large exon comprising the "ovarian cancer cluster region" (OCCR) and other associations with breast cancer cluster regions (BCCR), for both *BRCA1* and *2,* speak to the challenge of genetic heterogeneity [31].

8.3.2 *TP53*: Li–Fraumeni Syndrome

The *TP53* germline mutations give rise to Li–Fraumeni syndrome (LFS), a rare inherited cancer predisposition syndrome associated with approximately 1% of breast cancer cases. Germline mutations in this gene predispose to a wide spectrum of malignancies, including sarcomas, brain tumors, adrenocortical carcinomas, and leukemias, occurring at any point in an individual's lifetime, with a median age at diagnosis of first malignancy of 25 [32]. Otherwise, somatic *TP53* mutations are the most common mutations in adult adenocarcinomas.

TP53 is a tumor suppressor gene located on chromosome 17p13.1 that plays a major role in the regulation of cell growth [33]. Approximately 70% of patients with classic LFS criteria have a detectable TP53 germline mutation [34]. Mutations are most commonly missense, but deletions of the coding or promoter region of p53 can also occur [35].

TP53 mutation carriers face a lifetime risk of cancer that exceeds 90% [36]. Breast cancer is the most frequent malignancy among female *TP53* mutation carriers and represents up to one third of all cancers in LFS families [37]. Overall, although LFS is responsible for a small fraction of breast cancer cases, a woman with LFS has a breast cancer risk of 56% by the age of 45 and greater than 90% by the age of 60, which accounts for a 60-fold increased risk for early-onset breast cancer when compared to the general population [38, 39]. Women with LFS-related breast cancer have a tendency to present at a very young age (20s or 30s) with a more advanced disease (tumor > 5 cm, positive axillary nodes) [40–42]. Furthermore, recent studies have shown that two thirds of LFS-associated breast cancer tumors are positive for epidermal growth factor receptor 2 (HER2/neu) and/or estrogen and progesterone receptor [41, 42]. It is possible that the outcome of LFS patients identified in the modern era will be better because of the introduction of therapies that effectively target HER2.

Recently, with clinical availability of NGS-based multigene panel tests that analyze dozens of hereditary cancer genes in parallel usually including TP53, new challenges arise due to many patients without criteria for LFS being tested [43]. This less strict approach to genetic evaluation has resulted in the identification of mutations in various established hereditary cancer genes in patients who lack the expected phenotype, raising important questions about prevalence, penetrance, and phenotypic spectrum [44–46]. This technology has also enabled the identification of low-level DNA variation consistent with germline mosaicism or somatic interference, which can be particularly challenging in clinical practice [47].

8.3.3 *CDH1*: Hereditary Diffuse Gastric Cancer Syndrome

E-Cadherin germline mutations are responsible for the development of hereditary diffuse gastric cancer (HDGC), an autosomal inherited syndrome [48]. These constitutional alterations were first identified in a Maori population with a remarkable clustering of diffuse gastric cancer in a single large kindred [49]. This large pedigree was characterized by the presence of multiple gastric tumors as well as lobular breast cancers (LBCs) among female family members. A germline mutation in *CDH1* was identified among affected relatives. The *CDH1* gene is located on chromosome 16q22.1 and encodes the E-cadherin protein [50], which is critical for establishing and maintaining polarized and differentiated

epithelia through intercellular adhesion complexes, functioning as a cell invasion suppressor. Aberrant E-cadherin activity leads to loss of cell adhesion, increased cell motility, and metastatic ability of the tumor [51, 52].

The penetrance of gastric cancer in people with *CDH1* mutations is reported to be 70% for men and 56% for women by age 80. Furthermore, the cumulative risk of LBC for women with a *CDH1* mutation is estimated to be 42% by age 80. There is currently no evidence that the risk of other cancer types in individuals with a *CDH1* mutation is significantly increased [53].

Apart from the well-documented association between LBC and HDGC syndrome, novel E-cadherin germline mutations have recently been detected in individuals without history of HDGC. Recent studies have provided evidence that early-onset LBC might be the first manifestation of HDGC. Benusiglio et al. [54] identified E-cadherin germline deleterious mutations in three bilateral LBC cases (age at onset >50 years) not fulfilling the International Gastric Cancer Consortium criteria, negative at the beginning for HDGC in first- and second-degree relatives and without *BRCA1* and *BRCA2* alterations. Interestingly enough, E-cadherin mutations have been identified in four bilateral early-onset LBCs (age at onset >50 years) with no family history of HDGC [55].

Recently the International Gastric Cancer Consortium has added a novel criterion, recommending genetic testing also in bilateral LBC patients or women with a family history of two or more cases of LBC (>50 years at onset) [53]. However, *CDH1* germline mutations have also been identified in isolated cases with age at onset >45 years [56].

In a recent study, penetrance data for *CDH1* mutation carriers has been updated based on affected individuals who presented clinically with HDGC or LBC, from 75 families with pathogenic *CDH1* mutations. The cumulative risk of HDGC for *CDH1* mutation carriers by age 80 is reported to be 70% for men (95% CI 59–80%) and 56% for women (95% CI 44–69%). The cumulative risk of LBC for women with a *CDH1* mutation is estimated to be 42% (95% CI 23–68%) by age 80. There is currently no evidence that the risk of other cancer types in individuals with a *CDH1* mutation is significantly increased [57].

8.3.4 *PTEN*: Cowden Syndrome

Germline mutations in *PTEN* are the cause of Cowden syndrome (CS) or *PTEN* hamartoma tumor syndrome (PHTS). Hamartoma is a benign, focal malformation that resembles a neoplasm in the tissue of its origin. This is not a malignant tumor, and it grows at the same rate as the surrounding tissues. It is composed of tissue elements normally found at that site, but growing in a disorganized mass.

CS is an autosomal dominant multisystem disorder characterized by increased risks of malignant and benign tumors of the breast, thyroid, endometrium, and other organs, as well as a combination of mucocutaneous findings such as trichilemmomas, oral papillomas, and acral keratoses [58]. PHTS can be differentiated from other hereditary cancer syndromes based on personal as well as family history. However, many of the benign features of CS are common in the general population, making the diagnosis of CS challenging [59].

More than 90% of CS individuals with germline (heritable) *PTEN* mutations are believed to manifest some feature of the syndrome, although rarely cancer, by age 20, and by age 30, nearly 100% of mutation carriers are believed to have developed at least some of the mucocutaneous signs. CS remains underdiagnosed because of its variable expression (often with only subtle skin signs); consequently, the current prevalence estimate of one in 200,000 is still likely to be an underestimate [60].

PTEN is a phosphatase and tensin homolog located on chromosome 10q23.3 with phosphatidylinositol-3-kinase (PI3K) phosphatase activity. *PTEN*'s precise function is not clear; however, dysfunctional *PTEN* leads to the inability to activate cell cycle arrest and apoptosis, leading to abnormal cell survival [61]. Approximately 80% of affected individuals will have a detectable *PTEN* mutation that may include a missense, point, deletion, insertion, frame shift, or nonsense mutation [62]. Among the 20% of patients with no identifiable *PTEN* mutation, half may bear a mutation in *PTEN* promoter [63].

What is histologically unique in patients with CS is ductal adenocarcinoma surrounded by hyalinized collagen, and this suggests a diagnosis of CS. Women with CS also have a high risk (67%) of benign breast disease, such as fibroadenomas, microcysts, adenosis, and apocrine metaplasia. Mammary hamartomas are characteristic of this group of patients and might be multiple and bilateral. Colocalization with breast cancer is frequent [64]. In patients with germline *PTEN* mutations and thus PHTS, three studies to date have examined risks for malignancy [65–67]. The largest, by Tan et al., identified greatly increased lifetime risks for breast, thyroid, renal, and endometrial cancers and slightly elevated risks for colorectal cancers and melanoma [65].

Early estimates of breast cancer risk for females with *PTEN* mutations were traditionally reported to be around 25–50% [68, 69]. More recent studies have reexamined the lifetime risks for malignancy in patients with germline *PTEN* mutations and have found that early risk figures may have been underestimates [65–67, 70]. The largest of the three studies by Tan et al. identified increased risks for several types of cancer, with the highest risk estimate increase for female breast cancer. Tan et al. [68] identified an 85%

lifetime risk, beginning around age 30, for female breast cancer with 50% penetrance by age 50. This risk figure is comparable to that quoted for patients with HBOC syndrome [67] but has been controversial.

According to NCCN guidelines, the presence of a known *PTEN* mutation in an individual's family is a clear indication for genetic testing for CS. Genetic testing for CS is also warranted when several diagnostic criteria are met (Table 8.1) [71], which are mainly based on clinical phenotype and the development of neoplasia. The *PTEN* risk calculator was developed by the team at the Cleveland Clinic to evaluate patients with suspected CS and is available on their website. This tool was developed from a multicenter prospective study in which 3042 probands satisfying relaxed CS clinical criteria were accrued, and it can help to distinguish patients more likely have clinical CS and test positive for PTEN mutations [68]. This tool was also proven to be cost-effective and provided a well-calibrated estimation of pretest probability of *PTEN* status [60].

Table 8.1 National comprehensive cancer network 2015 Cowden syndrome criteria [71]

Major criteria
Breast cancer
Endometrial cancer (epithelial)
Thyroid cancer (follicular)
Gastrointestinal hamartomas (including ganglioneuromas but excluding hyperplastic polyps)
Lhermitte–Duclos disease (adult)
Macrocephaly (97th percentile: 58 cm for adult women, 60 cm for adult men)
Macular pigmentation of the glans penis
Multiple mucocutaneous lesions (any of the following):
Multiple trihilemmomas (3, at least 1 proven by biopsy)
Acral keratoses (3 palmoplantar keratotic pits and/or acral hyperkeratotic papules)
Mucocutaneous neuromas (3)
Oral papillomas (particularly on the tongue and gingival), multiple OR biopsy-proven OR dermatologist diagnosed
Minor criteria
Autism spectrum disorder
Colon cancer
Esophageal glycogenic acanthosis
Lipomas
Intellectual disability (i.e., intelligence quotient/75)
Renal cell carcinoma
Testicular lipomatosis
Thyroid cancer (papillary or follicular variant of papillary)
Thyroid structural lesions (e.g., adenoma, multinodular goiter)
Vascular anomalies (including multiple intracranial developmental venous anomalies)
Three or more major criteria, but one most include Lhermitte–Duclos disease, macrocephaly or GI hamartoma
Two major criteria plus three minor criteria

8.3.5 *STK11*: Peutz–Jeghers Syndrome

Germline mutations in the *STK-11* gene are the cause of Peutz–Jeghers syndrome (PJS). PJS is a rare autosomal dominant disorder characterized by multiple gastrointestinal hamartomatous polyps and mucocutaneous pigmentations of the lips, buccal mucosa, and digits. These lesions fade during puberty, with the exception of those in buccal mucosa. Polyps can occur anywhere in the gastrointestinal tract and can increase in size enough to cause bowel obstruction, most commonly in the small bowel [72].

The *STK-11* gene is located on chromosome 19p13.3 and encodes for serine–threonine protein kinase 11. It is designated as a tumor suppressor gene, participating in membrane bonding and apoptosis [73]. Furthermore, it is a negative regulator of the mTOR pathway [74]. Although PJS has been described since 1949 [75], *STK-11* mutations were identified as its cause in 1998 [76]. Mutations of *STK-11* are detected in approximately 70–80% of patients with PJS, with 15% of them being deletions [77].

Affected individuals are at increased risk for colorectal, breast, small bowel, pancreatic, gastric, and ovarian cancer. Women with PJS present with an increased risk for breast cancer that reaches 50% lifetime. Breast cancer can occur early, but at a lower incidence compared to LFS and CS. In a case series that included 240 patients with PJS, breast cancer incidence has been shown to rise up to 32% by the age of 60, whereas it was only 8% by the age of 40 [78]. Similar to the general population, breast cancer in individuals with PJS is usually ductal in histology. Interestingly, women with PJS also have a 20% risk for ovarian cancer, mainly sex cord tumors [79].

8.4 Moderate-Penetrance Genes

Following the discoveries of *BRCA1* and *BRCA2*, many additional genes have been identified as breast cancer susceptibility genes. A prominent group of these are referred to as moderate-risk susceptibility genes because protein-truncating variants and severely dysfunctional missense substitutions in them appear to confer, on average, two- to fivefold increased risk of breast cancer. This magnitude of increased risk is less dramatic than risks conferred by most pathogenic alleles in the high-risk genes *BRCA1*, *BRCA2*, and *PALB2*, but potentially high enough to influence the medical management of carriers [80]. However, unlike *BRCA1* and *BRCA2*, the risk these genes pose is less certain, although data are accumulating more rapidly because more testing is being done. The most important genes in this group, involved in breast cancer risk, are *CHEK2*, *ATM*, *PALB2*, *NF1*, *BARD1*, *BRIP1*, *MRE11A*, *NBN*, *RAD50*, *RAD51C*, and *RAD51D*.

8.4.1 CHEK2

CHEK2 gene encodes for a serine–threonine kinase, which is activated in response to DNA double-strand breaks. It has also been found to phosphorylate *BRCA1*, facilitating its roles in DNA repair [81]. Certain pathogenic *CHEK2* mutations have been associated with breast cancer. Mutation 110delC has been shown to increase breast cancer risk two- to threefold, while missense mutations have conferred lesser risk [82]. Histologically, 70–80% of *CHEK2*-associated breast cancers are ER-positive [83].

The *CHEK2* 1100delC mutation is particularly frequent in Northern European populations, where it confers a lifetime risk of breast cancer as high as 37% [84]. Homozygotes have a sixfold increased risk of breast cancer [58]. Additionally, some data suggest that *CHEK2* mutation carriers who develop breast cancer have a higher risk of recurrent breast cancers and a poorer disease outcome than noncarriers [85]. Although responsible for less than 1% of familial breast cancer syndromes, *CHEK2* mutations have been identified in approximately 5% of breast cancer patients who are not from BRCA breast cancer families. Furthermore, four other mutations of the *CHEK2* gene have been identified and appear to also confer a moderate breast cancer risk; however only limited data for these four variants are available [86].

8.4.2 ATM

Homozygous *ATM* mutation carriers suffer from ataxia-telangiectasia (AT), a disorder characterized by cerebral ataxia, immunodeficiency, and increased risk of certain malignancies, including breast cancer [87]. Heterozygous carriers of *ATM* mutations have a twofold increased breast cancer risk compared to general population. Women under age 50 with specific *ATM* mutations may have as high as a fivefold increased risk [88]. The risks are particularly difficult to assess because of the high frequency of ATM mutations in the general population.

ATM is a multifunctional gene that plays a pivotal role in double-strand break repair and in cell cycle progression. Genetic testing of *ATM* should be performed in members of families with a known mutation or a history of a clinical diagnosis of ataxia-telangiectasia. ATM is present on most multigene breast cancer susceptibility panels, so will typically be examined in patients with a history of hereditary breast cancer. However, clinical utility of *ATM* genetic testing in heterozygotes is difficult to assess and there are no specific guidelines at this time. Small studies have not demonstrated increased risk of radiation-induced second primary breast cancers, but definitive data on radiation sensitivity are not yet available. Of note, decreased expression of *ATM* protein has been associated with aggressive features in sporadic breast cancer [89].

8.4.3 PALB2

PALB2 has emerged a new breast cancer susceptibility gene that is in transition from moderate to high risk. It was named as a "binding partner and localizer of *BRCA2*," contributing to the DNA repair mechanism homologous recombination and tumor suppression [90]. Classification of *PALB2* as a breast cancer susceptibility gene was based on data showing that about 1% of individuals with hereditary breast cancer negative for *BRCA1/2* harbor a monoallelic mutation in *PALB2* [91]. In a recent study with data from approximately 1500 patients with familial breast cancer, the prevalence of *PALB2* mutations was 0.8%, with the majority occurring in high-risk patients [92]. Although the above studies characterize *PALB2* as a rare, intermediate-risk gene with regard to inherited genetic susceptibility to breast cancer, a recent study that included 154 families with *PALB2* mutations demonstrated a breast cancer risk of approximately 35% [93]. This estimated risk is higher than the one associated with other genes such as *CHEK2* and *ATM* and is classified as high, which may warrant the addition of *PALB2* genetic testing to *BRCA1/2* as a high-penetrance gene for breast cancer, particularly triple-negative disease.

8.4.4 NF1

Neurofibromatosis type 1 is an autosomal dominant tumor predisposition gene with a prevalence as high as one in 2000 births. The pleiomorphic condition is caused by mutations of the *NF1* gene on chromosome 17.3 [94]. *NF1* is a multisystem disease with varying combinations of benign and malignant tumors, developmental dysplasias, and functional deficits, including cognitive impairment. Almost all adult patients with *NF1* have cutaneous neurofibromas, which are benign tumors that do not become malignant. More than one half of patients with *NF1* also have plexiform neurofibromas, which may become malignant [95, 96]. The most common malignancies associated with *NF1* are intracranial gliomas and malignant peripheral nerve sheath tumors (MPNSTs) [97]. In addition to malignancies originating from the nervous system, other cancers associated with *NF1* include breast cancer, gastrointestinal stromal tumor (GIST), and pheochromocytoma [98–100]. Multiple population-based studies have demonstrated a three- to fivefold increase in lifetime breast cancer risk for women with *NF1*, with the highest risks for those <50 years of age. In a study with data from England, the age-specific excess risk of breast cancer comparing the *NF1* cohort with the control cohort was elevated 6.5-fold (95% confidence interval 2.6–13.5) in women aged 30–39, and there was a 4.4 (2.5–7.0) times higher risk among women aged 40–49 [101].

8.4.5 *NBN*, *RAD51C*, *RAD51D*, *BRIP1*, *RAD50*, and MRE11

Genes involved in the Fanconi anemia (FA)-BRCA pathway, critical for DNA repair by homologous recombination, interact in vivo with *BRCA1* and/or *BRCA2* [102]. Some of these genes are mainly associated with ovarian cancer rather than breast cancer, and data are still emerging.

In *NBN*, one protein-truncating variant, c.657del5, is sufficiently common in some Eastern European populations to allow its evaluation in a case–control study. A meta-analysis of ten studies reported strong evidence of an association with breast cancer risk for this variant (summary relative risk, 2.7; 90% CI, 1.9–3.7; $P = 5 \times 10^{-7}$) [103].

For two other DNA repair genes, *MRE11A* and *RAD50*, which encode proteins that form an evolutionarily conserved complex with *NBN*, the data is more conflicting [102, 104, 105]. Currently, there are insufficient data to consider them as breast cancer risk genes [106].

8.5 Multigene Panel Testing

Some considerations must be done when doing multigene panels:

Multipanel can include moderate-penetrance genes for which there are no clear guidelines on risk management for carriers of pathogenic mutations. Until data are clearer, identification of these mutations may not alter the management plan compared to what might be recommended based on family history alone, so their immediate clinical utility could be questioned.

The use of NGS panel testing will lead to a considerable increase in the finding of variants of uncertain significance (VUS), sequence alterations that may or may not affect the function of the gene, or its resultant protein. The frequency of VUS in a large series of clinical specimens examined with NGS breast cancer susceptibility panel reached about 40%, with up to five variants found in individual patients, depending on the series evaluated [11]. An uncertain result—a VUS can ultimately be reclassified as pathogenic or benign—can be very stressful for the patient and family. VUS are clinically troubling for several reasons, including the temptation to assume that a particular VUS is responsible for disease risk in a family, when most will ultimately be considered benign. They are inherited like any other sequence alteration, so it should be shared among family members, but few families are of sufficient size to allow for definitive classification of pathogenicity based on the association with disease status [107]. However, a fraction of VUS will be reclassified to disease causing, highlighting the need for providers to track VUS reclassification and inform patients, which requires time and resources often for many years. Multiple expert groups use functional laboratory assays and computational approaches to classify sequence alterations, which are maintained in publicly supported databases like ClinVar, ClinGen, and the new BRCA Challenge of the Global Alliance for Genomics and Health.

There is a chance of finding a gene that does not match with personal and family history. In some series this finding varies from 0.3 to 0.8% of the tests [11]. Here, the difficulty occurs when mutations are discovered in genes that are predicted to be unrelated to the clinical presentation (e.g., a 40-year-old woman with ductal carcinoma of the breast with no family history of gastric cancer and a mutation in *CDH1*). The appropriateness of counseling this young woman to consider risk-reducing gastrectomy or testing family members for the CDH1 mutation in the setting of concern for gastric cancer risk remains difficult to determine [44].

8.6 Management of Carriers of Mutations in High-Penetrance Genes

8.6.1 BRCA1 and BRCA2

The main goal in management of BRCA mutation carriers is to reduce the risk of developing cancer or at least to promote an early opportune diagnosis and increase the chances of cure.

8.6.1.1 Screening

Breast Cancer

Surveillance of breast cancer in BRCA carriers includes monthly self-examinations, clinical breast examinations twice a year, and yearly magnetic resonance imaging (MRI) of breasts starting at age 25–30 with the addition of annual mammograms thereafter. Earlier screening can be discussed in a family with history of breast cancer prior to age 30. Between ages 25 and 30, MRI is preferred over mammography as false-negative mammogram has been associated with dense breast tissue, and multiple prospective trials have demonstrated far inferior sensitivity of mammogram compared to MRI in BRCA1/2 mutation carriers. In *BRCA1*, the development of "interval cancers" between imaging studies led to the practice of alternating mammograms and MRI's 6 months apart and the recommendation for breast self-exam in *BRCA1* mutation carriers [108]. Between ages 30 and 75, at this time, both breast annual MRI and mammogram are recommended, and after age 75, screening must be individualized [71].

Although earlier studies have not shown an association between radiation exposure and an increased risk of breast cancer in *BRCA* carriers, a recent study did find an increased risk of breast cancer when patients are exposed

to radiation (including mammogram) before age 30. This study further highlights the possible advantage of using MRI alone in this group [109].

Ovarian Cancer

Unfortunately, there is no effective screening for ovarian cancer at this time. The use of transvaginal ultrasound plus CA 125 has not proven to be sufficiently sensitive and specific to substitute for surgery in women at increased genetic risk of ovarian and related cancers.

The NCCN does not consider screening for ovarian cancer to be a reasonable substitute for salpingo-oophorectomy in women with HBOC syndrome [34]. A woman who declines salpingo-oophorectomy can undergo screening with the use of serum measurement of CA 125 and transvaginal ultrasonography every 6–12 months starting at ages 30–35 or 5–10 years before the earliest diagnosis of ovarian cancer in the family, but the patient must be advised about the lack of evidence about this strategy [8]. Ongoing clinical trials are examining bilateral salpingectomies for ovarian cancer risk reduction, with plans for oophorectomies at natural menopause to avoid the very significant side effects of early surgical menopause. Long-term data on efficacy are not yet available.

Other Tumors

Patients with *BRCA1/2* mutations should undergo annually skin examinations as suggested in NCCN and ESMO guidelines because of increased risk of melanoma. There are no official guidelines about pancreatic screening, but the CAPS trials are available for individuals who carry a pathogenic *BRCA1/2* mutation and have a family history of pancreatic cancer in close relatives [8, 71].

8.6.1.2 Risk-Reduction Surgeries

Bilateral Mastectomy

Bilateral mastectomy is largely accepted as an option for women carrying a mutation in either *BRCA1* or *BRCA2*. From 1999 through 2004, the results of four retrospective and prospective observational studies were published. These studies compared breast cancer outcomes in women who underwent prophylactic mastectomy with women of similar risk who did not undergo surgery [110–114]. Four studies showed a **reduction of 90%** or more in the risk of subsequent breast cancer among women who underwent prophylactic mastectomy; updated reports and additional studies have confirmed these initial results.

Although bilateral mastectomies have shown to reduce breast cancer incidence, this procedure is not associated with reduction in breast cancer mortality. This information must be discussed with patients in order to help them decide between surgery and surveillance [113].

Recently, a new technique called nipple-sparing or total skin-sparing mastectomy is becoming more and more common. During this procedure, the surgeon preserves the overlying skin of the nipple areola complex and removes the underlying glandular tissue at risk. Reconstruction can be performed immediately with a variety of techniques. In this procedure, cosmesis is enhanced by preserving the nipple skin, leading to less psychosocial impact, but still loss of sensation and erectile function. More about these surgical options will be addressed in Chap. 10 [115].

Risk-Reducing Salpingo-Oophorectomies

Due to the lack of effective screening for ovarian cancer, *BRCA1/2* mutation carriers are advised to undergo a risk-reducing salpingo-oophorectomy (RRSO) between ages 35 and 40, after women have completed childbearing. This surgery reduces the risk of developing ovarian cancer by as much as 85–96% and breast cancer by approximately 50% [116–119] and reduces both ovarian and breast cancer mortalities [119]. For women with *BRCA2* mutations, who have previously undergone mastectomy, the delay of RRSO until ages 40–45 can be considered since the ovarian cancer onset tends to be late in *BRCA2* carriers [120]. Uptake of RRSO, however, varies widely among individuals and across countries, with lower uptake among European than American women in most data.

Premature surgically induced menopause can lead to an increased risk of cardiovascular disease, osteoporosis, vasomotor symptoms, sleep disturbances, mood swings, and sexual dysfunction and thus adversely affect quality of life. Hormone replacement therapy (HRT) has been shown to at least partially alleviate vasomotor symptoms in *BRCA1/2* mutation carriers and decrease fracture risk in the general population [121–123]. Data have shown that short-term use of HRT in *BRCA1/2* mutation carriers does not negate the breast cancer risk reduction gained by RRSO [124]. The use of HRT may therefore mitigate the adverse effects of surgery on quality of life.

Given the adverse effects of premature menopause and data that ovarian cancer most often originates in the fallopian tube fimbria rather than ovarian surface epithelium, there has been growing interest in salpingectomy with or without delayed oophorectomy as a risk-reducing strategy. It is important to recognize that even with a better profile of impact in quality of life, this approach is not the standard of care of risk-reducing surgery. Fortunately, clinical trials are under way [125, 126]. In addition, oophorectomy in premenopausal women has been shown to reduce the risk of developing breast cancer by 50% in BRCA carriers, depending on the patient age at the time of the procedure, and salpingectomy probably will not have the same effect. These data have recently been questioned.

Chemoprevention

Oral contraceptives, which reduce ovarian cancer risk in the general population, also reduce ovarian cancer risk in *BRCA1/2* mutation carriers, but aspects of the data have been controversial. Observational studies have shown associations between the use of oral contraceptives and a reduced risk of ovarian cancer among *BRCA1* and *BRCA2* carriers, with odds ratios suggesting a 40–50% reduction in risk [127, 128]. However, there are data showing a 2.5-fold increase in breast cancer risk in *BRCA2* carriers in particular, for whom the ovarian cancer lifetime risk is lower (10–20%). The risk reduction with oral contraceptives is generally not considered sufficient to render risk-reducing salpingo-oophorectomies unnecessary, though some women may feel more comfortable delaying surgery if they have used oral contraceptives for many years.

Currently, data on the use of tamoxifen for primary prevention of breast cancer in *BRCA1* and *BRCA2* carriers are very limited. The only prospective data derive from the National Surgical Adjuvant Breast and Bowel Project P1 trial where investigators identified mutation status in 288 women who developed breast cancer, among whom only eight *BRCA1* carriers and 11 *BRCA2* carriers were identified. The hazard ratios for the development of breast cancer among women who received tamoxifen were 1.67 (95% CI, 0.32–10.7) among *BRCA1* carriers and 0.38 (95% CI, 0.06–1.56) among *BRCA2* carriers. Although these results are limited by small sample sizes, they are consistent with an effect in *BRCA2* carriers; approximately 77% of breast cancers in *BRCA2* carriers are ER-positive. Because of small sample sizes, these results are uninformative for *BRCA1* carriers. And the major question of whether or not tamoxifen can provide primary prevention of breast cancer in *BRCA1* carriers, of whom 75–80% of breast cancers are ER-negative, remains. The case–control studies involving *BRCA1* and *BRCA2* carriers reported that tamoxifen protects against contralateral breast cancer with odds ratio of 0.50 (95% CI, 0.30–0.85) to 0.38 (95% CI 0.19–0.74) for *BRCA1* carriers and 0.42 (95% CI, 0.17–1.02) to 0.63 (95% CI 0.20–1.50) for *BRCA2* carriers [129, 130]. Data are more consistent for tamoxifen in the setting of secondary prevention.

There are some preclinical studies suggesting that the use of PARP inhibitors can delay tumor development and extend the life span of *BRCA1*-deficient mice [131], but there are no current trials in humans as these drugs have only limited approval in the therapeutic setting at this time.

8.7 Li–Fraumeni Syndrome

Current practice guidelines established by the NCCN recommend yearly physical examinations including skin and neurologic examinations for all individuals with LFS, with special attention to the possibility of rare malignancies, secondary malignancies, and/or pediatric cancers, depending on the at-risk population. The NCCN also advise some specific considerations as outlined in detail below.

8.7.1 Breast Cancer

Breast cancer is one of the most common cancers occurring among women with germline TP53 mutations. Breast cancer surveillance programs for women with LFS are based on data that established the value of breast MRI in women with *BRCA* mutations.

The complete screening program includes a clinical breast examination once or twice yearly beginning at the age of 20–25. This can be performed earlier depending on the earliest age of onset of breast cancer in the family. Imaging should be used as a screening modality annually starting at the age of 20–25, or earlier, depending on family history. The NCCN recommend that between ages 20 and 29, patients should receive annual MRIs; after age 29, patients should begin mammograms and continue with MRI [71]. European guidelines generally do not include mammography because of concerns about radiation exposure in this population.

There are no data on this specific group regarding risk-reducing surgery, but depending on the prevalence of breast cancer in LFS patients, mastectomy may be considered based on BRCA patient data [71, 132].

8.7.2 Whole-Body MRI

Regarding the large spectrum of tumor in patients with LFS, other strategies have been discussed in order to improve surveillance in this group. A recent study incorporated whole-body MRI into a comprehensive screening protocol that also included clinical examinations and laboratory measures [133]. Participants who elected to enroll in this enhanced screening protocol were compared with those who decided not to undergo screening. The group without screening presented with tumors when they became symptomatic. The striking finding was the difference in outcome between the two groups: individuals in the group who underwent screening had a significant survival advantage with 100% survival at 3 years compared to 21% in the non-surveillance group (95% CI 4–48%) [133]. Separate breast MRI is still required in females because whole-body MRI does not visualize the breasts in sufficient detail.

The use of MRI in this setting has the distinct advantage of avoiding ionizing radiation, and as technology improves, faster whole-body screens have become possible. The existing data, while impressive and hopeful, are neither randomized nor complete; thus whole-body MRI is not yet a standard

of care worldwide. However, multiple research centers are currently working toward the design and implementation of prospective whole-body MRI protocols for LFS families that will contribute to further our understanding regarding the risks and benefits of such screening. Currently, two trials in particular must be considered: the SIGNIFY study in the UK and the LIFESCREEN study in France. Carriers of germline *TP53* mutations should be encouraged to participate in such clinical trials as they are critical for identifying the best care and management strategies for individuals with LFS [132].

8.7.3 Other Tumors

The NCCN also advise consideration of colonoscopy every 2–5 years beginning at age 25 for LFS patients. Annual dermatologic exams are also recommended. Biochemical screening per the Toronto protocol for screening has been less widely adopted [133].

8.8 Cowden Syndrome

8.8.1 Screening

8.8.1.1 Breast Cancer

Women should start MMG and consider MRI, especially in the presence of dense breast tissue, by age 35 or 10 years before the earliest case in the family [71, 134, 135].

8.8.1.2 Other Tumors

As mentioned previously, all these screening procedures are based on expert opinion and have become incorporated into guidelines [71, 134] (Table 8.2). In particular, thyroid surveillance is noninvasive and has been widely adopted.

8.8.2 Risk-Reduction Surgery

8.8.2.1 Mastectomy

Given the high lifetime cancer risks of breast cancer to be 85% [65], prophylactic mastectomy can be discussed on an individual basis, particularly if breast imaging and clinical surveillance are challenging due to extensive benign breast involvement [71, 134].

Table 8.2 Screening procedures for other tumors related to Cowden syndrome

Cancer type	Recommended screening guidelines
Thyroid	Annual ultrasound
Endometrial	Starting at age 30: annual endometrial biopsy or transvaginal ultrasound
Renal cell	Starting at age 40: renal imaging every 2 years
Colon	Starting at age 35: colonoscopy every 2 years
Melanoma	Annual dermatologic examination

8.8.2.2 Hysterectomy

Hysterectomy should be discussed after childbearing, due to the risk of endometrial cancer [71, 135].

8.9 Peutz–Jeghers

8.9.1 Screening

The new guideline from the American College of Gastroenterology suggests that surveillance in affected or at-risk PJS patients should include monitoring for colon, stomach, small bowel, pancreas, breast, ovary, uterus, cervix, and testes cancers, but the guideline does not specify the frequency or the exams that should be performed [136]. Regarding breast cancer risk development, MRI should be considered, but mastectomy is not usually recommended [71].

8.10 Management of Carriers of Mutations in Moderate-Penetrance Genes

There is a lack of evidence regarding management of moderate-penetrance genes. Although these genes are more frequently evaluated by multipanel testing, for the great majority of them, there are no prospective good data about penetrance, management, and clinical utility [71, 137]. The cancer risks associated with mutations in these genes are lower and different than those reported for high-penetrance genes, and the extrapolation of guidelines for the management of individuals with high-penetrance variants of cancer susceptibility genes to the clinical care of patients with moderate-penetrance gene mutations could result in substantial harm [106].

The NCCN guidelines suggest to do breast MRI and/or mammogram. Breast MRI is recommended when lifetime breast cancer risk exceeds 20%, as in patients harboring mutations in *ATM*, CHECK2, and *PALB2*; there are insufficient data for the intervention for *BRIP1*. The guidelines suggest offering RRSO for patients with mutations in *BRIP1*, *RAD51C*, and *RAD51D*; there are insufficient data for *PALB2*. Mastectomy should be offered just to *PALB2*, and the evidence is still insufficient for *ATM* and CHECK2. Table 8.3 summarizes this discussion [71].

Another important consideration is when to start breast cancer screening for these patients. In a recent publication in *Nature Reviews Oncology*, Tung et al. discussed this issue and concluded that screening should begin in this population when the average 5-year lifetime risk exceeds population

Table 8.3 Clinical management guidelines of high-penetrance genes

Gene syndrome	Lifetime risk of breast cancer	Other tumors	Breast cancer screening	Risk-reducing surgery	Other screening
BRCA1/2 HBOC syndrome	50–85%	Ovarian, pancreatic, melanoma, prostate	25–29 MRI 30–75 MMG + MRI	Mastectomy RRSO	–
TP53 Li–Fraumeni syndrome	65–90%	Sarcoma, leukemia, adrenocortical brain tumors, other	20–29 MRI 30–75 MMG + MRI	Mastectomy	Whole-body MRI
CDHI HDGC syndrome	45%	Gastric	30–75 MMG + MRI	Mastectomy Gastrectomy	–
PTEN Cowden syndrome	85%	Endometrial thyroid, colorectal, renal	30–75 MMG + MRI	Mastectomy Hysterectomy	Colonoscopy Thyroid USG TVUS + Endo BX Renal Ultrasound
STK11 Peutz–Jeghers	32%	Colorectal, small bowel, pancreatic, gastric, and ovarian	30–75 MMG + MRI	–	–

Table 8.4 Screening guidelines for moderate-penetrance genes (after Tung et al. [106])

Gene	Mammography (clinical breast examination and/or MRI)	RRSO
ATM	Annual starting at 40 y	Based on family history
CHECK2 truncating	Annual starting at 40 y	Based on family history
NBN	Annual starting at 40 y	Based on family history
PALB2	Annual starting at 30 y	Based on family history
BRIP1	Based on family history	50–55 y
RAD51C	Based on family history	50–55 y
RAD51D	Based on family history	50–55 y

risk at ages 45–50, the age at which mammographic screening is recommended in women in the USA [106]. Women with pathogenic mutations in *PALB2*, *ATM*, *NBN*, and *CHEK2* (other than p.I157T) have a cumulative life risk (CLTR) of breast cancer that exceeds 20% and thus meet existing guidelines for MRI surveillance, at least in the USA. For practical reasons it would be reasonable to initiate MRI surveillance at the same time as mammography [106].

The suggested age to start screening annually with mammogram and/or MRI is 40 years for *ATM*, CHECK2, and *NBN* and 35 years for *PALB2*. The RRSO procedure should be considered only for *BRIP1*, *RAD51C*, and *RAD51D* between ages 50 and 55 [106] (Table 8.4).

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