Hereditary breast cancer: from molecular pathology to tailored therapies

D S P Tan, C Marchio, J S Reis-Filho

ABSTRACT
Hereditary breast cancer accounts for up to 5–10% of all breast carcinomas. Recent studies have demonstrated that mutations in two high-penetrance genes, namely BRCA1 and BRCA2, are responsible for about 16% of the familial risk of breast cancer. Even though subsequent studies have failed to find another high-penetrance breast cancer susceptibility gene, several genes that confer a moderate to low risk of breast cancer development have been identified; moreover, hereditary breast cancer can be part of multiple cancer syndromes. In this review we will focus on the hereditary breast carcinomas caused by mutations in BRCA1, BRCA2, Fanconi anaemia (FANC) genes, CHK2 and ATM tumour suppressor genes. We describe the hallmark histological features of these carcinomas compared with non-hereditary breast cancers and show how an accurate histopathological diagnosis may help improve the identification of patients to be screened for mutations. Finally, novel therapeutic approaches to treat patients with BRCA1 and BRCA2 germ line mutations, including cross-linking agents and PARP inhibitors, are discussed.

Family history is one of the most powerful risk factors for breast cancer development. It is estimated that approximately 5–10% of all breast cancers may be caused by mutations in high-penetrance susceptibility genes. Initial studies suggested that the vast majority of multiple-case breast cancer families and families with breast and ovarian cancer would be caused by mutations in BRCA1 or BRCA2 genes. However, more recent analyses have suggested that these initial studies may have overestimated the prevalence of BRCA1/BRCA2 mutations in hereditary breast cancer cases; in fact, mutations in these genes account for approximately 16% of the familial risk of breast cancer.

Subsequent studies have failed to find another high-penetrance breast cancer susceptibility gene (ie, a “BRCA3” gene), but several genes that confer low or moderate risk of breast cancer development have been identified. Genes that cause hereditary breast cancers can be broadly classified according to the level of risk they confer (table 1). Interestingly, from a functional perspective, most of the genes whose pathogenic mutations are associated with increased risk of breast cancer development are involved in DNA repair.

In addition, hereditary breast cancer may also be part of multiple cancer syndromes (eg, TP53 mutations in Li–Fraumeni syndrome, STK11/LKB1 mutations in Peutz–Jeghers syndrome; PTEN mutations in Cowden disease), which will not be reviewed here and are summarised in table 2. From a pathologist’s perspective, it should be noted that pathogenic mutations in the gene that encodes E-cadherin (CDH1) have been shown to be associated with the development of invasive lobular carcinoma. This is one of the best examples of genotypic–phenotypic correlations in hereditary breast cancer.

Interestingly, from a functional perspective, most of the genes whose pathogenic mutations are associated with increased risk of breast cancer development are involved in DNA damage signalling or repair. The loss of DNA repair is a crucial step for tumour cells to acquire genomic instability. Genomic instability refers to a tumour cell’s ability to undergo chromosomal rearrangements resulting in the formation of fusion oncogenes and inactivation of tumour suppressor genes, and amplify molecular drivers of tumour progression including oncogenic anti-apoptotic, cell-proliferative and drug-resistance genes. Clearly, genomic instability can only occur in tandem with a tolerance to DNA damage. In cancer cells, this can be achieved via loss of DNA damage signalling pathways and checkpoints such as those regulated by TP53 and ataxia telangiectasia mutated (ATM) proteins, or by loss of DNA repair pathways such as homologous recombination (HR). In the context of hereditary breast cancer, the most commonly mutated genes are BRCA1 and BRCA2, both of which are key players in HR DNA repair.

The focus of this review will be on hereditary breast cancer caused by mutations in BRCA1, BRCA2, Fanconi anaemia (FANC) genes, CHK2 and ATM tumour suppressor genes. Gene polymorphisms recently identified in genome-wide single nucleotide polymorphism (SNP) arrays are beyond the scope of this review, and interested readers should refer to recent reviews on this topic (see Stratton and Rahman and references therein).

HEREDITARY BREAST CANCER GENES
BRCA1 and BRCA2
Linkage analysis studies initially identified a gene on 17q that was associated with cases of early onset breast cancer, but it was Miki and colleagues who cloned BRCA1 back in 1994. Following its cloning, BRCA1 functions, mutations and the risk conferred by BRCA1 germ line mutations have been extensively studied.

The BRCA1 gene encodes a 1863-amino-acid protein that has several domains, including a ring-finger domain, a nuclear localisation signal, DNA-binding domain, SQ cluster domains and Breast Cancer Gene 1 (BRCA1) carboxyl-terminal domain (BRCT) domains. The SQ cluster region is the site of phosphorylation by ATM and ATR during S-phase, and the BRCT is a region comprising ~100
amino acid tandem repeats at the C-terminus of the BRCA1 protein which may have a direct role in the regulation of DNA-damage responses, cell cycle checkpoints, or DNA repair. Single or multiple BRCT motifs are also present in many other proteins involved in DNA-damage checkpoint control and DNA repair. The majority of BRCA1 mutations result in truncated protein products that lack one or both C-terminal BRCT domains, and loss of this region leads to tumour formation in mice. In addition, clinically relevant missense mutations at the C-terminus of BRCA1 lead to disruption of the BRCT structure.

Owing to its size and number of distinct domains, it is not surprising that BRCA1 has numerous functions, the best characterised of them being related to the role this protein plays in homologous recombination DNA repair, cell cycle checkpoint control, ubiquitylation, chromatin remodelling and DNA decatenation. In addition, BRCA1 also plays a role in the transcriptional regulation of certain genes, including oestrogen receptor.

Linkage analysis and positional cloning studies led to the identification of BRCA2 in 1995 using familial breast cancer pedigrees with multiple cases of breast cancer in successive generations. This gene encodes a protein that is even larger than BRCA1, but its functions seem to be more limited than those of BRCA1: DNA repair, cytokinesis and meiosis. It is currently accepted that BRCA1 and BRCA2 are essential for accurate repair of DNA double-strand breaks by homologous recombination repair.

**Table 1** Genes whose mutations are reported to cause increased risk of hereditary breast cancers, classified according to the level of risk they confer

<table>
<thead>
<tr>
<th>Breast cancer risk</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk: 10- to 20-fold relative risk</td>
<td>BRCA1 (17q21)</td>
</tr>
<tr>
<td></td>
<td>BRCA2 (13q12.3)</td>
</tr>
<tr>
<td></td>
<td>TP53 (17p13.1)</td>
</tr>
<tr>
<td>Intermediate risk: two- to fourfold relative risk</td>
<td>CHEK2 (22q12.1)</td>
</tr>
<tr>
<td></td>
<td>ATM (11q22.3)</td>
</tr>
<tr>
<td></td>
<td>CDH1 (16q22.1)</td>
</tr>
<tr>
<td></td>
<td>PTEN (10q23.31)</td>
</tr>
<tr>
<td></td>
<td>BRIP1/FANCl (17q22)</td>
</tr>
<tr>
<td></td>
<td>PALB2/FANCN (16p12)</td>
</tr>
<tr>
<td>Possible low risk: &lt;twofold</td>
<td>FANCl (16q24.3)</td>
</tr>
<tr>
<td></td>
<td>FANCe (6p22-p21)</td>
</tr>
</tbody>
</table>

**Table 2** Summary of the syndromes associated with hereditary breast cancer

<table>
<thead>
<tr>
<th>Syndrome (OMIM)</th>
<th>Gene involved and cytoband</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary Breast Cancer and ovarian cancer syndrome (113705)</td>
<td>BRCA1 (17q21)</td>
<td>Breast cancer, high risk (50–60%)</td>
</tr>
<tr>
<td>Hereditary Breast Cancer and ovarian cancer syndrome (600185)</td>
<td>BRCA2 (13q12.3)</td>
<td>Ovarian cancer, high risk (40–50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer, high risk (50–70%)</td>
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<tr>
<td></td>
<td></td>
<td>Ovarian cancer, intermediate risk (10%)</td>
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<tr>
<td></td>
<td></td>
<td>Prostate cancer</td>
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<td></td>
<td></td>
<td>Pancreatic cancer</td>
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<tr>
<td></td>
<td></td>
<td>Melanoma</td>
</tr>
<tr>
<td>CHEK2 mutations (Li–Fraumeni 2 syndrome?)</td>
<td>CHEK2 (22q12.1)</td>
<td>Breast cancer, intermediate risk (~twofold)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain tumours</td>
</tr>
<tr>
<td>Other FANC genes (114480, 610355, 607139, 600901, 605882)</td>
<td>PALB2/FANCN (16p12)</td>
<td>PALB2/FANCN and BRIP1/FANCl: moderate risk of breast cancer development</td>
</tr>
<tr>
<td></td>
<td>FANCl (16q24.3)</td>
<td>The other FANC genes: low risk of breast cancer development</td>
</tr>
<tr>
<td></td>
<td>FANCe (6p22-p21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRIP1/FANCl (17q22)</td>
<td></td>
</tr>
<tr>
<td>Familial-linitis-plastic type gastric cancer and lobular breast carcinomas syndrome (192080)</td>
<td>CDH1 (16q22.1)</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>Louis–Bar syndrome (208900)</td>
<td>ATM (11q22.3)</td>
<td>Lobular breast cancer</td>
</tr>
<tr>
<td>Li–Fraumeni syndrome (151623)</td>
<td>TP53 (17p13.1)</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Cowden syndrome (158350)</td>
<td>PTEN (10q23.31)</td>
<td>Cerebellar ataxia</td>
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<td></td>
<td></td>
<td>Immune deficiency</td>
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<td></td>
<td></td>
<td>Glioma</td>
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<td></td>
<td></td>
<td>Medulloblastoma</td>
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<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Bannayan–Riley–Rivalcaba syndrome (153480)</td>
<td>PTEN (10q23.31)</td>
<td>High penetrance for breast cancers at young age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Risk of soft-tissue sarcomas and osteosarcomas, brain tumours, leukaemia and adrenocortical carcinoma</td>
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<tr>
<td></td>
<td></td>
<td>Increased risk of developing neoplasms (breast cancer, thyroid carcinoma, endometrielial carcinoma and others)</td>
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<tr>
<td></td>
<td></td>
<td>Hamartomatous polyps of the gastrointestinal tract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muco-cutaneous lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meningioma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follicular cells tumours of the thyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melanocytic macules of the lips, buccal mucosa and digits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple gastrointestinal hamartomatous polyps</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased risk of various neoplasms (breast, testis, pancreas and cervix)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased risk of endometrial carcinoma and colorectal carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High risk of multiple primary malignant neoplasms, including breast, ovarian, gastrointestinal and genitourinary carcinomas, sarcomas, gliaoblastoma and leukaemia</td>
</tr>
</tbody>
</table>
Several studies have now demonstrated that pathogenic mutations (ie, either truncating mutations or mutations that cause nonsense-mediated RNA decay) of BRCA1 and BRCA2 genes are strongly associated with early-onset breast cancer.\(^3\)\(^,\)\(^4\) These mutations confer a 10- to 20-fold increased risk of breast cancer development, resulting in up to 70–80% risk of breast cancer development by the age of 70.\(^5\) In addition, mutations in these genes are associated with increased risk of ovarian cancer, predominantly of the serous papillary type, but endometrioid and clear cell can also be found.\(^6\)\(^,\)\(^7\) Interestingly, site-specific ovarian cancer has been associated with missense mutations in BRCA2, and a higher risk of ovarian cancer relative to breast cancer has been observed with mutations on a segment of exon 11 of BRCA2, a 3.5 kb region known as the ovarian cancer cluster region.\(^8\) BRCA1 mutations are also associated, to a lesser extent, with other types of cancer, including papillary serous carcinoma of the peritoneum and prostate cancer. Pathogenic BRCA2 gene mutations are also associated with a 5–7% risk of breast cancer development in men.\(^9\) The pathogenicity of rare missense substitutions or frame exon deletions in BRCA1 and BRCA2 (known as unclassified variants) remains unclear and continues to present a challenge for genetic counsellors in the clinical setting. However, a recent study of unclassified variants incorporating oestrogen receptor, cytokeratin 5/6, and cytokeratin 18 correlates with tumour markers of BRCA1 mutation status suggests that the BRCA1 variants IVS18+1G>T (del exon 18) and 5562 T>A (VI138E) may be pathogenic.\(^10\)

Animal models demonstrate that complete loss of function of BRCA1 is embryonic lethal,\(^11\) whereas BRCA2 homozygous mutations are associated with Fanconi anaemia and the development of familial medulloblastoma and Wilms tumours.\(^12\)\(^,\)\(^13\) Despite the controversies about BRCA1 and BRCA2 haploinsufficiency, there are several lines of evidence to suggest that there is no functional consequence of heterozygous BRCA1 or BRCA2 mutations, until the wild type BRCA1 or BRCA2 allele is lost, mutated or silenced.\(^14\) It has been shown to be an early feature of neoplastic cells of BRCA1 or BRCA2 mutation carriers.\(^15\) It is currently believed that the loss of conservative DNA repair and genome stability functions due to BRCA1 or BRCA2 inactivation in the premalignant tissues drives the rapid acquisition of additional mutations, as evidenced by the profound chromosomal instability of BRCA1 and BRCA2 associated breast and ovarian cancers when compared with sporadic cases.\(^16\) In contrast, normal tissues of BRCA1 or BRCA2 mutation carriers are heterozygous for the mutation, retain a wild type allele and therefore maintain normal or near-normal gene function.

### FANC genes

Fanconi anaemia, a rare recessive genetic disorder, is characterised by skeletal abnormalities, short stature, microphthalmia and abnormal skin pigmentation. This disease is known to be caused by homozygous mutations in a number of genes (see below).\(^2\) Interestingly, cells of patients with Fanconi anaemia have an exquisite sensitivity to mitomycin C or diepoxybutane. When exposed to these drugs, cells from patients with Fanconi anaemia show a marked increase in the frequency of chromosomal breaks compared with normal cells.\(^2\)\(^,\)\(^3\) This characteristic is rather similar to the level of chromosomal instability found in BRCA2 knockout mice.\(^2\) However, it was only in 2002 that bilallelic mutations of BRCA2 were shown to cause Fanconi anaemia.\(^2\)

An initial study suggested that mutations in Fanconi anaemia genes other than BRCA2 (FANCD1) would not be associated with increased risk of breast cancer.\(^2\) The same group, however, demonstrated in a substantially larger study that truncating FANCJ (BRIP1) mutations are associated with a low, but significant increase in the risk of breast cancer development.\(^6\) Since then, PALB2, another gene that causes Fanconi anaemia when completely inactivated (ie, homozygous inactivating mutations), has also been shown to cause breast cancer when a heterozygous (mono-allelic) inactivating mutation is found.\(^6\)\(^,\)\(^7\)

Currently, the list of genes of this family reported to confer increased risk of breast cancer when mutated in germ line DNA include FANCD1 (aka BRCA2), FANCJ (aka BRIP1) and FANCN (aka PALB2).\(^6\)\(^,\)\(^7\) The main function of these genes is related to homologous recombination DNA repair, and cancer cells with loss of function of these genes have both a remarkable level of genomic instability and an exquisite sensitivity to cross-linking agents (fig 5).\(^6\)\(^,\)\(^7\)

Although mutations in other FANC genes have not been reported in the context of hereditary breast cancer, there is indirect evidence to suggest that these genes may be inactivated in both sporadic and familial cancers through epigenetic/transcriptional mechanisms (eg, FANCN2 is downregulated at the immunohistochemical level about 20% and 10% of sporadic and hereditary breast carcinomas, respectively).\(^6\)

### CHK2

CHK2 encodes a protein kinase that has been shown to be an important signal transducer of cellular responses to DNA damage and a candidate tumour suppressor. CHK2 is involved in cell-cycle checkpoint control by phosphorylating Cdc25 phosphatases, leading to their subsequent degradation, and activating p53 and BRCA1. Hence, this protein is either directly or indirectly involved in multiple cellular functions, including cell-cycle control, apoptosis, and DNA repair. Furthermore, CHK2 is activated in the presence of DNA damage by ATM kinase. Although CHK2 gene germ line mutations were initially believed to cause Li–Fraumeni and Li–Fraumeni-like syndrome, more recent studies have called into question the associations between CHK2 mutations and these syndromes.\(^8\) CHK2 truncating mutations have been shown to confer a moderate risk of breast cancer development. Meijers-Heijboer et al\(^9\) found that 1100delC CHK2 truncating mutation results in an...
approximately twofold increase in breast cancer risk in women and a 10-fold increase in risk in men. Apart from the 1100delC, other founding mutations have been described. The 1157T (470T>G) variant has been found in Finland population to confer an absolute risk of breast cancer of 8.1% in carriers by age 70 years, compared with 5.5% for non-carriers. Similar frequencies have been reported in Polish, German and Byelorussian populations, whereas population studies in the UK, North America and Netherlands demonstrated that frequencies between patients and controls groups were quite similar. The IVS2 +1 G>A splicing mutation is associated with a two- to fourfold elevated risk for breast cancer in Polish and Byelorussian populations, while in the Ashkenazi Jewish population two novel amino-acid substitutions have been found, S423F (1265C>T) and P95L (284C>T). S423F carriers were shown to have a twofold increase in breast cancer risk, whereas frequencies for P95L did not show any difference between cases and controls. A novel 5.6 kb genomic deletion has been discovered in two families of Czechoslovakian ancestry. Other rare variants have been found as well, but their significance have been found as well, but their significance in terms of breast cancer risk is yet to be determined.40

**ATM**

Ataxia telangiectasia is a rare, recessive autosomal disorder characterised by increased genetic instability, radiosensitivity, neurodegeneration, oculocutaneous telangiectasia, immune defects and cancer predisposition.40 Patients with this syndrome have homozygous ATM gene mutations.40 Like the above breast-cancer-related genes, ATM encodes a protein kinase that plays an important role in DNA repair following genetic insults that lead to DNA double-strand breaks. In the presence of double-strand breaks, ATM phosphorylates BRCA1 and p53.40 There are conflicting data on ATM and breast cancer predisposition, but there are data to suggest that the mutations that cause ataxia telangiectasia in biallelic mutation carriers overall confer an approximately twofold increased risk of breast cancer development in monoallelic mutation carriers.2 In addition, analysis of the association between other types of ATM mutations and the risk of breast cancer development has rendered inconclusive results.2

**Other genes**

NBS1 encodes a protein that is a member of the MRE11/RAD50 complex, which consists of five proteins (ie, NBS1, p200, p400, MRE11 and RAD50) and is involved in DNA double-strand break repair and DNA damage-induced checkpoint activation. Homozygous mutations of NBS1 cause the Nijmegen breakage syndrome, an autosomal recessive syndrome characterised by chromosomal instability, microcephaly, growth retardation, immunodeficiency and cancer predisposition.40 The estimated prevalence of the most common pathogenic NBS1 gene mutation (657del5) is approximately 1.3×10^7 persons, and the proportion of breast cancer attributable to this mutation is less than 1%.40-47 In fact, the breast cancer risk conferred by NBS1 mutations is estimated to be low.40-41

Interestingly, with other genes that would theoretically be good candidates for breast cancer risk mutations, such as RAD50, ATR, CHK1, PP2R1B, PP2R5B and ELF2b/Int-6,40-44 there is no evidence to support the association of truncating mutations of these genes and significantly higher risk of breast cancer development.

**PATHOLOGY OF HEREDITARY BREAST CANCER**

Since the cloning of BRCA1 and BRCA2, the pathological and molecular features of tumours arising in mutation carriers have been extensively studied.1-3 This is by no means an academic exercise only, given that referring a patient to genetic counselling and BRCA gene mutation analysis is currently based on algorithms that largely rely on family history and patients’ characteristics.

Although these algorithms have acceptable levels of sensitivity, their specificity is clearly suboptimal.41 There are data to suggest that based on the current algorithms for genetic testing, >20% of routine patients attending a multidisciplinary breast cancer clinic would have a probability sufficiently high by at least one algorithm to be offered genetic testing.41 Furthermore, more recent population-based studies have suggested that the reliance of the current genetic algorithms on family history may also be problematic, as up to 9.5% of women with BRCA1 or BRCA2 mutations and breast cancer diagnosed before the age of 50 do not have any obvious history of familial early-onset breast cancer or familial ovarian cancer.41

It is currently accepted that BRCA1 mutation carriers are characterised by a constellation of morphological, immunohistochemical and molecular features that are distinct from those of BRCA2 mutated cancers and age-matched sporadic controls.42-47 On the other hand, although BRCA2 carriers64-65 and non-BRCA1/BRCA266 familial breast cancers have several phenotypic differences when compared with controls, these are not sufficient to allow for the identification of these cancers with a significant degree of certainty.45,47

The analysis of the prevalence of specific histological types of tumours arising in BRCA1 and BRCA2 germ-line mutation carriers has revealed that the majority of these cancers are invasive ductal carcinomas of no special type, but medullary breast carcinomas are more often found in BRCA1 carriers (11%) than in controls or BRCA2 carriers.1,46-55 On the other hand, there is some evidence to suggest that BRCA2 cancers may more often be of tubular, tubulo-lobular, lobular and pleomorphic lobular morphology when compared with controls.1,58-62 However, the largest study on BRCA2 mutated cancers to date failed to identify this association.52 Studies on the prevalence of in situ disease in BRCA1 and BRCA2 cancers compared with controls by different groups have yielded conflicting results.46-52 Data on ductal carcinoma in situ (DCIS) in the context of BRCA1 and BRCA2 cancers are contentious. While the Breast Cancer Linkage Consortium observed a significantly lower prevalence of DCIS in BRCA1 mutation carriers,63 others65 have observed a similar frequency. Interestingly, Hwang et al,41 found that DCIS in BRCA1 mutation carriers was significantly more often of high grade when compared with DCIS in non-carriers; on the other hand, no statistically significant difference was seen between DCIS in BRCA2 mutation carriers and non-carriers.

Detailed morphological analyses of BRCA1 mutation carriers have demonstrated that these tumours are characterised by high histological grade, high mitotic counts, solid sheets of tumour cells, conspicuous nucleoli, brisk lymphocytic infiltrate, continuous pushing borders/margins and an increased frequency of necrotic areas when compared with BRCA2 mutation carriers and sporadic age-matched controls.1,16,45,50,55,63,65 Multivariate analysis revealed that of the above morphological features, only high mitotic counts, pushing borders and lymphocytic infiltrate were significantly associated with a BRCA1 genotype, whereas the differences in the morphological features of BRCA2 cancers
and age-matched controls were not so striking. In fact, only a higher degree of tubule formation, lower mitotic counts and continuous pushing borders were found to be associated with BRCA2 cancers.46 On the other hand, other studies,15 35 including a more recent and larger case control study of BRCA2 mutation carriers and controls, failed to confirm the association of most pathological features and BRCA2 germ line mutations, apart from the higher prevalence of pushing borders.41 In fact, Bane et al35 have demonstrated that tumours arising in BRCA2 mutation carriers are significantly associated with grade III features including reduced tubule formation, a higher mitotic score and nuclear pleomorphism.51

The expression of hormone receptors seems to be different in BRCA1, BRCA2 and sporadic controls. A large, logistic regression analysis revealed that lack of oestrogen receptor (OR) expression is the strongest predictor of BRCA1 germ line mutation.39 It has been reported that 65–90% of all BRCA1 cancers lack of OR expression,1 15 47 52 54 55 but this seems to vary according to age at diagnosis. One could argue that lack of OR expression in BRCA1 cancers could be a mere reflection of the high percentage of grade III cancers, but when BRCA1 cancers and grade-matched controls were compared, the likelihood of a grade III BRCA1 cancer to be OR negative is 4.8 x that of a sporadic, grade-matched control.63 Similar associations have been found between BRCA1 mutations and progesterone receptor expression (PR).1 15 47 53 54 55 On the other hand, the prevalence of OR expression in BRCA2 cancers seems not to differ from those of controls.15 47 54 Interestingly, in a study where the prevalence of OR expression in BRCA2 carriers was compared with that of grade-matched controls, a significantly higher prevalence was found in BRCA2 cancers.81

Studies have shown that HER2 overexpression is uncommon in both BRCA1 and BRCA2 cancers when compared with controls,15 47 54 but conflicting results are on record due to different antibodies and scoring systems used. In studies where the Herceptest scoring system was employed, it was observed that 0–3% of BRCA1 cancers and approximately 6% of BRCA2 tumours are HER2-positive. Similar results have been reported for HER2 gene amplification.64 66 Several reasons for the lack of HER2 gene amplification in BRCA1 and BRCA2 cancers have been put forward, including codetection of BRCA1 and HER2 loci in BRCA1 cancers, distinct mechanisms of genetic instability or different cells of origin.1 15

Our group15 60 and others67 have shown that tumours arising in BRCA1 mutation carriers more often show a triple negative (OR-, PR- and HER2-) and basal-like phenotype than BRCA2 tumours and controls. The expression of basal markers, including cytokeratin (CK) 5/6, CK 14, CK 17, epidermal growth factor receptor (EGFR), P-cadherin, HIF-1α and caveolin 1, is significantly more frequent in BRCA1 cancers when compared with BRCA2 tumours and controls.15 47 60 71 Interestingly, recent studies have also highlighted the similarities between tumours arising in BRCA1 mutation carriers and sporadic basal-like breast cancers at the immunohistochemical and genomic/genetic levels45 60 72 75 and demonstrated that BRCA1 pathway is dysfunctional in the majority of sporadic basal-like cancers44 (see below).

TP53 gene mutations and p53 protein expression by immunohistochemical analysis have been shown to be more prevalent in BRCA1 and BRCA2 cancers when compared with controls. This gene is affected in 30–77% of all BRCA1 cancers and in 20–65% of all BRCA2 cancers.1 15 75 The lack of consistency in these results stems from different sequence strategies, and distinct antibodies and different cut-offs for immunohistochemical analysis. The importance of TP53 gene mutations is also corroborated by the results of BRCA1−/− animal models, which are embryonic lethal; however, lethality can be delayed by co-inactivation of p53.76 Interestingly, there are data to suggest that the pattern of TP53 mutations found in BRCA1 cancers differs from that found in sporadic breast carcinomas,75 but a direct comparison between the type of TP53 mutations in BRCA1 cancers and sporadic basal-like breast carcinomas,75 which have a similar prevalence of TP53 mutations when compared with BRCA1 cancers, is yet to be carried out.

There have been several reports highlighting the differences between the immunohistochemical profiles of BRCA1 cancers, including the analysis of proteins related to apoptosis and cell cycle control.15 54 55 64 Owing to the fact that the vast majority of BRCA1 cancers are OR-negative and the role played by BRCA1 in the regulation of OR expression, it is not surprising that BRCA1 cancers lack the expression of several genes associated with OR expression, such as bcl2 and cyclin D1.15 In fact, the pattern of expression of p27, cyclin E1 and other genes is remarkably similar to that found in basal-like breast cancers.15 In addition, amplification of CCND1 has been shown to be vanishingly rare in both BRCA177 and basal-like breast cancers75 but is found in >10% of BRCA2 and sporadic controls.80

From a molecular genetics perspective, again, BRCA1 cancers harbour patterns of chromosomal copy-number gains and losses that are distinctive from those usually found in sporadic controls, whereas the genetic profiles of BRCA2 cancers are similar to those of sporadic cancers.81–85 Interestingly, the genetic features reported for BRCA1 cancers are remarkably similar to those described for sporadic basal-like breast cancers.15 54 65

A recent study77 has described the pattern of expression of genes related to DNA repair in BRCA1, BRCA2 and sporadic controls, and demonstrated that in BRCA1 cancers, PCNA and CHK2 are overexpressed, and RAD50 is downregulated, whereas BRCA2 cancers also show downregulation of CHK2, but RAD50 is found at the same levels as those found in sporadic controls. That study also demonstrated that BRCA2 cancers more often show cytoplasmic expression of RAD51 than controls; this does make biological sense, as RAD51 nuclear foci formation requires a functional BRCA2. Although the results by Honrado et al86 are promising, independent validation of the results is yet to be published.

Taken together, it is clear that the morphological features of BRCA2 cancers are of limited help in identifying patients to be screened for mutations. Conversely, BRCA1 cancers are characterised by a rather specific constellation of morphological and immunohistochemical features. Histopathological and immunohistochemical models to predict BRCA1 germ line mutations have been developed. Farshid and colleagues68 proposed a system based on OR, PR and the above morphological features of BRCA1 tumours, which has a similar sensitivity when compared with clinical models, but much higher specificity (86%) and positive and negative predictive values (61% and 98%, respectively). In fact, an immunohistochemical predictor of BRCA1 germ line mutation using OR and CK5/6 has been shown to have a sensitivity of 56%, a specificity of 87% and positive and negative predictive values of 28% and 99%, respectively.89 Based on these lines of evidence, it seems clear that models incorporating clinical features, family history, histopathological features and immunohistochemical profiles of the tumours would be best suited for the identification of patients with BRCA1 mutations. Although further evidence in support of the predictive values of the aforementioned models is still required,
these findings can at least be used to help decide which gene should be tested in a patient with family history strongly suggestive of familial breast and ovarian cancer; if the tumour lacks OR expression and is positive for "basal" markers, BRCA1 rather than BRCA2 should be sequenced. 15-17

Another model incorporating both immunohistochemical and clinical details to identify BRCA1 related carcinomas has been proposed by van der Groep et al., 18-20 who were able to identify a "probably sporadic" class and a "probably BRCA1-related" class using a decision tree with age, Ki67 and EGFR. The "probably sporadic" class was defined by women aged >54 years old, affected by a tumour displaying a proliferative index ≤25% and negativity for EGFR; 79% of the sporadic cases fell into this class, whereas no BRCA1-related breast cancer fulfilled the criteria. The "probably BRCA1-related" class was defined by women aged ≤54 years old, affected by a tumour displaying a proliferative index ≥25% and positivity for EGFR, and comprised 82% of the BRCA1-related cases but only 1.4% of the sporadic cases. 60 Although these models are promising, validation in large cohort of patients with BRCA1 mutations needs to be performed.

It should be noted, however, that the distinctive morphological, immunohistochemical and molecular features of BRCA1 and BRCA2 cancers are significantly attenuated when tumours are diagnosed after 55 years of age. 15, 57, 58 Several hypotheses have been advanced to explain these differences, including the possibility of the development of a sporadic breast cancer in the context of a BRCA1 or BRCA2 germ line mutation, without inactivation of BRCA1 or BRCA2 wild type allele in cancer cells.

NON-BRCA1/BRCA2 BREAST CANCERS

The phenotypic characteristics of cancers developing in patients with a strong family history that do not have BRCA1 or BRCA2 germ line mutations have been comprehensively studied. It is currently accepted that these cases constitute a heterogeneous group of cancers, likely to be explained by a polygenic model due to the interaction of multiple low-penetrance genes. Non-BRCA1/BRCA2 familial cancers are of a lower histological grade than sporadic cancers, but their immunohistochemical profiles are rather similar to those of sporadic cancers. 43-46, 61 Furthermore, using an immunohistochemistry-based hierarchical clustering approach that is yet to be validated, Honrado et al. 60 demonstrated that non-BRCA1/BRCA2 cancers may be classified into the five main molecular subgroups previously identified by expression profiling analysis of breast cancera (ie, luminal A, luminal B, basal-like, normal breast-like and HER2). Interestingly, these authors have demonstrated that concurrent BRCA1 loss of heterozygosity and gene promoter methylation were preferentially found in non-BRCA1/BRCA2 with a basal-like phenotype. 60

With the increasingly more coherent data on genes whose mutations are associated with moderate risk of breast cancer and low-penetration breast cancer SNPs, it is possible that in the future, the genes underlying a significant proportion of this heterogeneous group of cancer will be identified.

CHK2

The analysis of the morphological features of tumours developing in patients with CHK2 gene mutations has yielded conflicting results. 16-18 While CHK2 cancers are reported to express OR more frequently than controls, results for PR have been more inconsistent. Furthermore, one of the founding mutations in the Polish population (U157T) has been reported to be associated with lobular carcinomas. 26

ATM

Given the rarity of ATM mutations in patients with breast cancer, there is a paucity of data on the phenotype of cancers arising in patients with ATM germ line mutations. A recent study by the KConFab group failed to identify any significant differences between the morphological features of tumours arising in patients with IVS106T->G, 2424V->G and 1420L->F ATM mutations and age-matched controls. 91

NOVEL THERAPEUTIC STRATEGIES FOR PATIENTS WITH HEREDITARY BREAST CANCER

Given the defects on homologous recombination DNA repair found in cancer cells with inactivation of BRCA1 and BRCA2, it is not surprising that preclinical models have demonstrated that these cells show an exquisite sensitivity to DNA cross-linking agents (eg, carboplatin, cisplatin and mitomycin-C). These agents cause genomic lesions that lead to a collapse of DNA replication forks, which subsequently require DNA repair by homologous recombination for fork repair and restart. Without functional BRCA1 and BRCA2, cells treated with cross-linking agents would have an overload of genetic rearrangements and eventually die due to mitotic catastrophe. 26 Cass et al. have previously demonstrated higher rates of tumour response to first-line platinum-based chemotherapy in Jewish BRCA mutation carriers with ovarian cancer compared with non-hereditary patients with ovarian cancer as well as a significant correlation between the in vivo and in vitro response of their tumours to platinum and paclitaxel. 49 In addition, low/intermediate levels of BRCA1 mRNA have recently been shown to confer a significantly improved overall survival following treatment with platinum-based chemotherapy in comparison with patients with high levels of BRCA1 mRNA. 60 On the other hand, the role played by taxanes for the management of BRCA1 and BRCA2 carriers is less clear. There are some data to suggest that BRCA1 loss of function may lead to microtubule stabilisation and resistance to paclitaxel, 16 which is supported by the observation that inhibition of endogenous BRCA1 expression in ovarian cancer cell lines results in increased platinum sensitivity and decreased sensitivity to antimicrotubule agents. 16 To answer the question of clinical taxol resistance in patients with BRCA1 or BRCA2 germ line mutations, a randomised phase II clinical study (BRCA trial, UK) will compare the efficacy of carboplatin and docetaxel in BRCA1 and BRCA2 carriers with advanced breast cancer. It is hoped that the results of this study will also provide direct evidence to either confirm or refute the hypothesis of a greater efficacy of cross-linking agents in patients with breast cancer with BRCA germ line mutations.

It has been demonstrated that inactivation of other mechanisms of DNA repair, in particular base excision and single-strand break-repair pathways, in cells that do not have functional homologous recombination DNA-repair mechanisms is lethal (ie, synthetic lethality). Given that BRCA1 and BRCA2 heterozygous mutations do not abrogate homologous recombination DNA repair, inhibition of other types of DNA repair would only be toxic in cells harbouring loss of function of BRCA1 or BRCA2 (ie, tumour cells) and not in normal cells (which would still have at least one functional copy of BRCA1 and BRCA2). 16, 17 17 In this context, drug inhibition of single-strand break-repair mechanism would only cause lethality in BRCA1 or BRCA2-deficient cancer cells. Poly-ADP Ribose Polymerase (PARP)
inhibitors have been shown to cause highly selective cell killing in cells that have lost function of BRCA196, 97 or BRCA2,98 as well as in cells with loss of function of other components of the BRCA1, BRCA2 and Fanconi network.96 For detailed reviews on this topic, the readers are recommended to refer to Ashworth100 and Shiu et al.101

Although there was initially some controversy regarding the level of sensitivity to PARP inhibitors that BRCA1 and BRCA2 mutations would confer,99 100 102 this has now been shown to be strongly associated with the potency of the PARP inhibitor,103 selective cell killing is only seen in drugs with an IC50 in the nanomolar range.104 Furthermore, data on the mechanism of resistance to PARP inhibitors and carboplatin in BRCA2 mutant cell lines and human cancers provide strong evidence in support of the role played by BRCA1 and BRCA2 inactivation in the sensitivity to these agents.104 Results of the single agent phase I clinical trial with the Kudos/Astra Zeneca compound AZD2281 are encouraging, with a favourable toxicity profile, pharmacodynamic evidence of PARP inhibition from the analyses of surrogate tissues and objective evidence of anti-tumour efficacy in patients with BRCA1 or BRCA2 ovarian cancers. Phase II trials of PARP inhibitors in BRCA1 and BRCA2 carriers with advanced breast cancer are under way.

ARE THERE ANY SPORADIC TUMOURS THAT RECAPITULATE THE CHARACTERISTICS OF BRCA1 OR BRCA2 GENES?

Unlike TP53, whose germ line mutations cause Li–Fraumeni syndrome and somatic mutations are often found in sporadic breast carcinomas, BRCA1 and BRCA2 somatic mutations are reported to be remarkably rare in sporadic breast cancers. However, other mechanisms leading to BRCA1 and BRCA2 inactivation may play a role in subgroups of sporadic cancers.9

BRCA1 gene silencing by DNA promoter methylation is reported to be found in 10–30% of sporadic breast cancers.105–107 Interestingly, BRCA1 gene promoter methylation has been shown to be associated with high histological grade, lack of oestrogen and progesterone receptors and medullary histological type—all features of BRCA1 cancers and sporadic basal-like breast carcinomas.15 However, a study comparing the prevalence of BRCA1 gene promoter methylation and the molecular subgroups defined by microarray analysis failed to find any association between BRCA1 gene promoter methylation and basal-like phenotype.108 Our group analysed the prevalence of BRCA1 gene promoter methylation in basal-like cancers, defined by the expression of basal keratins, and grade and age-matched controls and failed to find any correlation between basal-like phenotype and BRCA1 gene promoter methylation.109 However, we did find a strong correlation between expression of basal keratins and overexpression of ID4, a negative regulator of BRCA1 gene expression.110 In addition, a strong, inverse correlation between BRCA1 and ID4 mRNA levels was also observed.111 An analysis of metaplastic breast cancers, tumours that display a basal-like phenotype in >90% of cases,112 demonstrated that in >60% of these cancers, the BRCA1 gene is silenced by gene promoter methylation.113 Taken together, these data suggest that a significant proportion of sporadic breast cancers may have a dysfunctional BRCA1 pathway and that these cancers are predominantly of basal-like and triple negative phenotypes.

Apart from harbouiring a dysfunctional BRCA1 pathway, sporadic basal-like cancers also appear to recapitulate the histopathological features of BRCA1 cancers. As described above, BRCA1 and sporadic basal-like cancers have remarkably similar morphological features and immunohistochemical profiles.114 Furthermore, BRCA1 tumours have been shown to consistently segregate together with sporadic basal-like breast cancers in hierarchical clustering analysis using microarray expression profiling data.115 In addition, the pattern of genomic losses, gains and gene amplifications found in BRCA1 cancers is distinct from that of a non-phenotype-matched cohort of sporadic cancers and, not surprisingly, similar to that described in basal-like cancers.15 It should also be noted that, despite the issues related to the specificity of anti-BRCA1 antibodies110 and the significance of aberrant BRCA1 cytoplasmic localisation,111 there is evidence to suggest that BRCA1 is downregulated at the protein level in basal-like breast carcinomas.111

Based on the fact that the majority of basal-like breast cancers show a dysfunctional BRCA1 pathway and harbour TP53 gene mutations, our group has engineered the conditional mouse BLG-Cre;BRCA1f22–24/p53–/p53–−, where BRCA1 gene is inactivated in β-lactoglobulin-expressing cells (ie, luminal epithelial cells of the mouse mammary gland), and all cells of the animal have only one wild-type allele of p53.112 Consistent with the findings of human BRCA1 and basal-like cancers, pathological analysis of the tumours arising in the above mouse model revealed that 78% lacked hormone receptors and HER2, and expressed basal markers (ck 14 and/or EGFR), and 88% showed homologous metaplastic elements.113 This mouse model provides another line of evidence for the link between basal-like phenotype and BRCA1 pathway dysfunction and may prove useful for testing novel therapies for basal-like cancers. Subsequently, Liu et al engineered another conditional mouse model BRCA1f18–/f18–, p53f18–/f18– lineage ablation and p53f18–/f18– gene inactivation, which spontaneously develops tumours with morphological and phenotypic characteristics remarkably similar to those observed in our model, despite the fact that BRCA1 was inactivated in keratin 14-positive cells of the mouse mammary gland.113

Somatic inactivation of BRCA2, on the other hand, seems to be remarkably uncommon.114 Interestingly, there is some evidence to suggest that amplification of EMSY may be the somatic counterpart of BRCA2 inactivation.115 The EMSY gene encodes a 1522-amino-acid protein that has a unique N-terminal 30-amino acid domain that is conserved between several species. EMSY is reported to interact with BRCA2 by binding to a small epitope within the BRCA2 exon 8-encoded transcriptional activation domain.116 When EMSY binds to BRCA2, it silences its activation potential. In addition, EMSY has also been implicated in chromatin remodelling. EMSY is reported to be amplified in 18% of breast cancer cell lines and in up to 7.5–13% of sporadic breast cancers;115 116 this amplification has been shown to be found more frequently in OR-positive cancers and to be associated with poor prognosis.116 A study published in abstract form at the USCAP meeting in 2007 reported on the analysis of EMSY gene amplification in 64 BRCA2 cancers and 186 age-matched controls demonstrated 9% of EMSY gene amplification in sporadic cancers and in none of the BRCA2 tumours. It was suggested that BRCA2 mutations and EMSY gene amplification may be mutually exclusive.117 Further studies are required to confirm these findings.

CONCLUSION AND FUTURE PERSPECTIVES

BRCA1 breast cancers are characterised by a constellation of morphological, immunohistochemical and molecular features that may help to identify high-risk patients for BRCA1 mutation testing. Sporadic basal-like breast cancers seem to phenocopy BRCA1 cancers, and conditional engineered mouse models have subsequently confirmed the genotypic–phenotypic correlations between BRCA1 mutations and basal-like phenotype. On the other hand, the majority of BRCA2 cancers may be of the luminal B (non-A) phenotype (ie, OR-positive but of a
high grade), and although some phenotypic characteristics of BRCA2 cancers seem to differ from that of sporadic controls, these differences are not sufficient to accurately differentiate these tumours from controls. The morphological features of hereditary breast cancer caused by mutations of genes associated with a moderate risk of breast cancer do not seem to differ significantly from sporadic controls, but the numbers studied so far are rather small.

The realisation of the associations between impaired DNA repair mechanisms and breast cancer risk not only offers insights into the development of breast cancer, but also offers new therapeutic avenues that are currently being explored.

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