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Hereditary breast cancer: from molecular pathology to tailored therapies

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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 (*FANCF*) 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.^{1,2} 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.^{1,3} 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.^{4,5}

Subsequent studies have failed to find another high-penetrance breast cancer susceptibility gene (ie, a “*BRCA3*” gene),³ but several genes that confer low or moderate risk of breast cancer development have been identified.^{3,6} 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.⁶ 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–Jegher 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.⁷ 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.⁶ 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.⁸ 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).⁹ 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.⁹

The focus of this review will be on hereditary breast cancer caused by mutations in *BRCA1*, *BRCA2*, Fanconi anaemia (*FANCF*) 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 Rahman⁶ 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,¹⁰ but it was Miki and colleagues who cloned *BRCA1*,¹¹ 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.³ The SQ cluster region is the site of phosphorylation by *ATM* and *ATR* during S-phase,¹² and the BRCT is a region comprising ~100

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

Breast cancer risk	Genes
High risk: 10- to 20-fold relative risk	<i>BRCA1</i> (17q21)
	<i>BRCA2</i> (13q12.3)
	<i>TP53</i> (17p13.1)
Intermediate risk: two- to fourfold relative risk	<i>CHEK2</i> (22q12.1)
	<i>ATM</i> (11q22.3)
	<i>CDH1</i> (16q22.1)
	<i>PTEN</i> (10q23.31)
	<i>BRIP1/FANCI</i> (17q22)
	<i>PALB2/FANCN</i> (16p12)
Possible low risk: <twofold	<i>FANCA</i> (16q24.3)
	<i>FANCB</i> (6p22-p21)

-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.¹³ 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.¹⁴

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.^{3 15-17} In addition, *BRCA1* also plays a role in the transcriptional regulation of certain genes, including oestrogen receptor.^{3 15}

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.^{18 19} 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.¹⁷ It is currently accepted that *BRCA1* and *BRCA2* are essential for accurate repair of DNA double-strand breaks by homologous recombination repair.¹⁷

Table 2 Summary of the syndromes associated with hereditary breast cancer

Syndrome (OMIM)	Gene involved and cytoband	Clinical features
Hereditary Breast Cancer and ovarian cancer syndrome (113705)	<i>BRCA1</i> (17q21)	Breast cancer, high risk (50–80%) Ovarian cancer, high risk (40–50%)
Hereditary Breast Cancer and ovarian cancer syndrome (600185)	<i>BRCA2</i> (13q12.3)	Breast cancer, high risk (50–70%) Ovarian cancer, intermediate risk (10%) Prostate cancer Pancreatic cancer Melanoma
CHEK2 mutations (Li–Fraumeni 2 syndrome?)	<i>CHEK2</i> (22q12.1)	Breast cancer, intermediate risk (~twofold) Sarcomas Brain tumours
Other FANC genes (114480, 610355, 607139, 600901, 605882)	<i>PALB2/FANCN</i> (16p12) <i>FANCA</i> (16q24.3) <i>FANCB</i> (6p22-p21) <i>BRIP1/FANCI</i> (17q22)	<i>PALB2/FANCN</i> and <i>BRIP1/FANCI</i> : moderate risk of breast cancer development The other FANC genes: low risk of breast cancer development
Familial-linitis-plastic type gastric cancer and lobular breast carcinomas syndrome (192090)	<i>CDH1</i> (16q22.1)	Gastric cancer Lobular breast cancer
Louis–Bar syndrome (208900)	<i>ATM</i> (11q22.3)	Lymphoma Cerebellar ataxia Immune deficiency Glioma Medulloblastoma Breast cancer
Li–Fraumeni syndrome (151623)	<i>TP53</i> (17p13.1)	High penetrance for breast cancers at young age Risk of soft-tissue sarcomas and osteosarcomas, brain tumours, leukaemia and adrenocortical carcinoma
Cowden syndrome (158350)	<i>PTEN</i> (10q23.31)	Increased risk of developing neoplasms (breast cancer, thyroid carcinoma, endometrial carcinoma and others) Hamartomatous polyps of the gastrointestinal tract Mucocutaneous lesions
Bannayan–Riley–Rivalcaba syndrome (153480)	<i>PTEN</i> (10q23.31)	Breast cancer Meningioma Follicular cells tumours of the thyroid
Peutz–Jeghers syndrome (175200)	<i>STK11</i> (19p13.3)	Melanocytic macules of the lips, buccal mucosa and digits Multiple gastrointestinal hamartomatous polyps Increased risk of various neoplasms (breast, testis, pancreas and cervix)
Lynch cancer family syndrome II (114400)	<i>MSH2</i> (2p22-p21), <i>MSH3</i> (5q11-q12), <i>MSH6</i> (2P16), <i>MLH1</i> (3p21.3), <i>PMS1</i> (2q31-q33), <i>PMS2</i> (7p22)	Increased risk of endometrial carcinoma and colorectal carcinoma High risk of multiple primary malignant neoplasms, including breast, ovarian, gastrointestinal and genitourinary carcinomas, sarcomas, glioblastoma and leukaemia

Table 3 Genomic regions differentially gained and lost in *BRCA1* when compared with sporadic controls

	Wessels <i>et al</i> ⁶³	Van Beers <i>et al</i> ⁶²	Jonsson <i>et al</i> ⁶¹
Most frequent copy-number gains	3q	3pter-t22	1q42.12–q42.13
	7p	3q13–q27	3q26.32–q26.33
	8q	8p12–pcent	3q27.1–q27.33
	10p	10pter–p12	7q36.1–q36.3
	12p	16p	8q24.23–q24.3
	16p	18p	10p15.3
	17q		10p15.1–p14
Most frequent copy-number losses	3p	5cent–q23	4p15.32–p14
	4p		Q131.3
	5q		4q32.1–q34
	12q		5q11.2–q23.3
	16p		8pter–p12
	18q		13q13.3–q21.32
			15q12–q13.1
			15q15.3–q21.1
		17p13.2–p12	

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.^{3–6} 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 70.³ 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.^{20–21} 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 3.3 kb region known as the ovarian cancer cluster region.²² *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.³ The pathogenicity of rare missense substitutions or inframe 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 14 as tumour markers of *BRCA1* mutation status suggests that the *BRCA1* variants IVS18+1 G>T (del exon 18) and 5632 T >A (V1838E) may be pathogenic.²³

Animal models demonstrate that complete loss of function of *BRCA1* is embryonic lethal,²⁴ whereas *BRCA2* homozygous mutations are associated with Fanconi anaemia and the development of familial medulloblastoma and Wilms tumours.²⁵ 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.²⁶ This has been shown to be an early feature of neoplastic cells of *BRCA1* or *BRCA2* mutation carriers.²⁷ 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.³ 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.²⁸ However, it was only in 2002 that bi-allelic mutations of *BRCA2* were shown to cause Fanconi anaemia.²⁵

An initial study suggested that mutations in Fanconi anaemia genes other than *BRCA2* (*FANCD1*) would not be associated with increased risk of breast cancer.²⁹ The same group, however, demonstrated in a substantially larger study that truncating *FANCI* (*BRIP1*) mutations are associated with a low, but significant increase in the risk of breast cancer development.³⁰ 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.^{31–34} 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*), *FANCI* (aka *BRIP1*) and *FANCN* (aka *PALB2*).^{2–6} 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 3).^{2–6}

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, *FANCD2* is downregulated at the immunohistochemical level about 20% and 10% of sporadic and hereditary breast carcinomas, respectively).³⁵

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.³⁶ *CHK2* truncating mutations have been shown to confer a moderate risk of breast cancer development. Meijers-Heijboer *et al*³⁷ 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 I157T (470T>C) 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, S428F (1283C>T) and P85L (254C>T). S428F carriers were shown to have a twofold increase in breast cancer risk, whereas frequencies for P85L 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 in terms of breast cancer risk is yet to be determined.³⁶

ATM

Ataxia telangiectasia is a rare, recessive autosomal disorder characterised by increased genetic instability, radiosensitivity, neurodegeneration, oculocutaneous telangiectasia, immune defects and cancer predisposition.³⁸ Patients with this syndrome have homozygous *ATM* gene mutations.² Like the above breast-cancer-related genes, *ATM* encodes a checkpoint 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 p53.³⁸ 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.³⁹ The estimated prevalence of the most common pathogenic *NBS1* gene mutation (657del5) is approximately 1:3×10⁶ persons, and the proportion of breast cancer attributable to this mutation is less than 1%.⁴⁰⁻⁴² 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*, *PPP2R1B*, *PPP2R5B* and *EIF2S6/Int-6*,^{43 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 15 45-57} 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.⁵⁵ 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.⁵⁸ 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.⁵⁹

It is currently accepted that *BRCA1* mutation cancers 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.^{1 15 46 47 55 56} On the other hand, although *BRCA2*^{1 15 46 47 51 55 56} and non-*BRCA1/BRCA2*^{1 45 60 61} 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.^{1 55}

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 53} 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 53 62} However, the largest study on *BRCA2* mutated cancers to date failed to identify this association.⁵¹ Studies on the prevalence of *in situ* disease in *BRCA1* and *BRCA2* cancers compared with controls by different groups have yielded conflicting results.^{1 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 others⁵² have observed a similar frequency. Interestingly, Hwang *et al*,⁶⁴ 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 cancers 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 cancers and sporadic age-matched controls.^{1 15 46 50 53 56} 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,⁴⁶ 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,^{1 51} 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.⁵¹ In fact, Bane *et al*⁵¹ 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.⁴⁷ It has been reported that 63–90% of all *BRCA1* cancers lack of OR expression,^{1 15 47 52 54 61} 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× that of a sporadic, grade-matched control.⁶⁵ Similar associations have been found between *BRCA1* mutations and progesterone receptor expression (PR).^{1 15 47 52 54 61} On the other hand, the prevalence of OR expression in *BRCA2* cancers seems not to differ from those of controls.^{1 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.⁵¹

Studies have shown that HER2 overexpression is uncommon in both *BRCA1* and *BRCA2* cancers when compared with controls,^{1 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.^{61 66} Several reasons for the lack of *HER2* gene amplification in *BRCA1* and *BRCA2* cancers have been put forward, including codeletion of *BRCA1* and *HER2* loci in *BRCA1* cancers, distinct mechanisms of genetic instability or different cells of origin.^{1 15}

Our group^{15 48} 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-1 α and caveolin 1, is significantly more frequent in *BRCA1* cancers when compared with *BRCA2* tumours and controls.^{1 15 48 68–71} 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 levels^{15 60 72 73} and demonstrated that *BRCA1* pathway is dysfunctional in the majority of sporadic basal-like cancers⁷⁴ (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 20–63% 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 coinactivation of *p53*.⁷⁶ Interestingly, there are data to suggest that the pattern of *TP53* mutations found in *BRCA* 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,¹⁵ 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.^{1 54 56 61} 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 *BRCA1*⁷⁷ and basal-like breast cancers^{78 79} 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.^{81–83} Interestingly, the genetic features reported for *BRCA1* cancers are remarkably similar to those described for sporadic basal-like breast cancers.^{9 15 84 85}

A recent study⁵⁷ 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 al*⁵⁷ 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 colleagues⁵⁵ 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.⁴⁸ 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

Another model incorporating both immunohistochemical and clinical details to identify *BRCA1* related carcinomas has been proposed by van der Groep *et al*,⁸⁶ 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 $\leq 25\%$ 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 $\geq 25\%$ and positivity for EGFR, and comprised 82% of the *BRCA1*-related cases but only 1.4% of the sporadic cases.⁸⁶ 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 87 88} 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.^{1 45 60 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^{72 89} (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-penetrance breast cancer SNPs,⁶ 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.^{56 90} 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.⁹⁰

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.⁹² 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 vitro* and *in vivo* response of their tumours to platinum and paclitaxel.⁹³ 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.⁹⁴ 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,⁹⁵ 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.⁹⁴ 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*).^{92 96 97} 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 BRCA1^{98–99} 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,^{98–99–102} 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.¹⁰³ 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.⁷⁴ However, we did find a strong correlation between expression of basal keratins and overexpression of ID4, a negative regulator of *BRCA1* gene expression.⁷⁴ In addition, a strong, inverse correlation between BRCA1 and ID4 mRNA levels was also observed.⁷⁴ An analysis of metaplastic breast cancers, tumours that display a basal-like phenotype in >90% of cases,¹⁰⁹ demonstrated that in >60% of these cancers, the *BRCA1* gene is silenced by gene promoter methylation.⁷⁴ 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 harbouring 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.⁷⁴ 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.⁷² 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;*BRCA1*^{F22–24/F22–24};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.¹¹² 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 *BRCA1*^{F/F}; p53^{F/F}, 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 1322-amino-acid protein that has a unique N-terminal 80-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 3-encoded transcriptional activation domain.¹¹⁵ 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

Take-home messages

- ▶ BRCA1 cancers are characterised by a constellation of morphological, immunohistochemical and molecular features that can help triage patients who should be subjected to BRCA1 gene testing.
- ▶ BRCA1 cancers and sporadic basal-like breast carcinomas have overwhelmingly similar pathological and molecular features.
- ▶ Novel therapeutic strategies for the management of BRCA1 and BRCA2 cancers are emerging, including cross-linking agents and inhibitors of PARP1.
- ▶ Non-BRCA1/BRCA2 cancers constitute a heterogeneous group of cancers.
- ▶ Most genes whose germ line mutations are associated with low/intermediate-risk of breast cancer development are associated with DNA-repair mechanisms.

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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