BRCA1 AND *BRCA2*: 1994 AND BEYOND

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Abstract | The discovery of the first gene associated with hereditary breast cancer, *BRCA1*, was anticipated to greatly increase our understanding of both hereditary and sporadic forms of breast cancer, and to lead to therapeutic and preventive breakthroughs. Much has been learned during the past decade about the genetic epidemiology of breast cancer, the ethnic distribution and clinical consequences of *BRCA1* and *BRCA2* mutations, and the central role of DNA repair in breast cancer susceptibility. The ability to translate this knowledge into novel treatments, however, remains elusive.

A decade has passed since Mark Skolnick and his colleagues at Myriad Genetics in Salt Lake City (Utah, USA) announced in 1994 that they had cloned the *BRCA1* gene[1]. *BRCA1* had been named three years earlier by Mary-Claire King when she and her group assigned it to chromosome 17 by linkage analysis using a large group of families with cases of early-onset breast cancer[2]; however, the identification of truncating mutations in the coding sequence of *BRCA1* in families with multiple cases of breast cancer was the conclusive step[1]. Predictions were soon made about new biological insights and potential therapies. Families with a high incidence of male breast cancer, however, were found not to carry *BRCA1* mutations[3], leading to the search for other breast cancer genes. *BRCA2* was linked to chromosome 13 in 1994 (REF. 4) and was cloned only a year later by the same group[5] (BOX 1).

Genetic testing for cancer susceptibility quickly followed. Testing was initially viewed with a mixture of exuberance and caution. Fears were expressed by social scientists and feminists, and breast cancer advocates expressed concern about the possible negative consequences of testing and its rapid commercialization. They felt that scientists might be paying undue attention to inherited causes of cancer that were beyond the control of individual women and were therefore not amenable to public policy and legislation, as opposed to the elimination of environmental toxins and the promotion of a healthy lifestyle. Psychologists recommended that women should complete a psychological evaluation before undergoing genetic testing. Genetic counsellors advised that women should attend several comprehensive pre-test counselling sessions, where a host of possible concerns, such as insurance and occupational discrimination, would be discussed. Many researchers therefore concluded that genetic testing should only be carried out in research settings. In an editorial in the *New England Journal of Medicine* in January 1996, Francis Collins, Director of the National Center for Human Genome Research at the United States National Institutes of Health said "the uncertain risks and benefits lead most observers to believe that testing, whether in Jewish or non-Jewish women, should now be done only in a research setting, with a protocol approved by an institutional review board and full informed consent"[6].

In retrospect, few of these initial fears have been realized. Caryn Lerman studied the psychological consequences of genetic testing soon after it was introduced and reported that it did not lead to undue increases in anxiety or depression[7]. There is now compelling evidence that both preventive mastectomy and preventive oophorectomy can markedly reduce cancer risk[8,9]. In the past decade, women have been increasingly accepting of preventive surgery, although chemoprevention has remained unpopular. Kelly Metcalfe surveyed women who had recently received a positive result after genetic testing in North America and found that 60% underwent preventive oophorectomy and 25% opted for prophylactic mastectomy, whereas only 12% had taken tamoxifen. At present, genetic testing is offered in many centres in North America, Europe, Australia and Israel. Several mutation surveys have been conducted in Asian countries[10], but genetic testing for cancer is still mainly a feature only of western medicine. Whether or not

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Summary

- In the ten years since the discovery of *BRCA1* and *BRCA2*, genetic testing for breast and ovarian cancer susceptibility has become integrated into the practice of clinical oncology.
- Attempts to identify a third breast cancer susceptibility locus (*BRCA3*) have so far been unsuccessful. This is probably because no single gene can account for the remainder of families that show a high incidence of breast cancer that is not associated with *BRCA1* or *BRCA2*.
- In general, the genes that have been identified as being associated with hereditary breast cancer (*BRCA1*, *BRCA2*, *TP53*, *CHK2* and *ATM*) are involved in the maintenance of genomic integrity and DNA repair.
- The risk of developing cancer is not identical for all carriers of *BRCA1* and *BRCA2* mutations. Risk can be influenced by allelic heterogeneity, modifier genes, and environmental and hormonal cofactors.

genetic testing will be perceived to be useful in Asia, Africa and South America, where it would compete for scarce health-care resources, is a question for the next decade. Even in North America, interest in genetic testing is greater among white than non-white populations[11].

Because *BRCA* mutations occur at a frequency of about 1 in 250 women, there are probably 250,000 women in the United States who are carriers. However, it is likely that fewer than 10,000 of these women have been identified. This might be because of the perceived lack of effective preventive measures. It might also be the case that only a minority of women with mutations have a family history of cancer that is sufficiently strong to attract notice and result in referral to a genetic-testing centre. Some women might be reluctant to pursue testing because of concerns about confidentiality and discrimination, whereas others might not have access to testing facilities. But what has the hunt for breast-cancer-associated genes taught us about breast cancer itself?

BRCA1 and *BRCA2* mutations

To some extent, the types of mutation that have been reported reflect the ease with which they are detected and the unambiguous nature of their effects on the BRCA1 protein. For this reason, most of the mutations that were first reported result in protein truncations; these are either small insertions or deletions, or are nonsense mutations that lead to the introduction of a stop codon[1,12–14]. These mutations invariably generate a shortened, non-functional BRCA1 protein.

Box 1 | ***BRCA* mutations in women with breast and ovarian cancer**

Studies indicate that it is worthwhile to screen all patients with invasive ovarian cancer or certain types of breast cancer, as more than 10% of tests will identify a *BRCA1* or *BRCA2* mutation (see table below).

Group	Proportion with *BRCA* mutations
Women with invasive ovarian cancer (all ages)	12%
Jewish women with breast cancer (all ages)	11%
Families with two or more cases of breast cancer in women under 50 years of age	12%

The Breast Cancer Information Core (BIC; see online links box) was established in 1995 to catalogue the range and frequency of germline *BRCA1* and *BRCA2* mutations. Because of the preponderance of protein-truncating mutations, the research community was quick to adopt the *in vitro* translation technique — also known as the protein-truncation test (PTT) — which was adapted by Hogervorst in 1995 (REF. 15). The test was quick and inexpensive, and reagents were available in kit form. PTT is still widely used to detect *BRCA1* and *BRCA2* mutations. The main limitation of PTT is that it is not effective for screening small exons using genomic DNA samples. Some laboratories have restricted its use to the screening of large exons of *BRCA1* (exon 11) and *BRCA2* (exons 10 and 11), and other laboratories have adapted it for the screening of cDNAs that are generated by reverse transcription. To ensure a comprehensive and sensitive 'gold standard', Myriad Genetics developed a robotic sequencing technique to screen for mutations on a commercial basis. Other more rapid and less expensive techniques have been developed to identify mutations[16–18] and, in general, these perform well[19,20] (TABLE 1).

If a family contains more than two cases of early-onset breast cancer and at least two cases of ovarian cancer, it is likely that a *BRCA* mutation will be found[21]. However, in most families, it is only possible to identify *BRCA1* or *BRCA2* mutations from a small proportion of women who receive genetic counselling. Factors that predict that a mutation will be found include the number of affected relatives who have breast or ovarian cancer, their ages at the time of diagnosis of breast cancer (but not ovarian cancer), Jewish ancestry and certain pathological features of the cases of breast and ovarian cancer that have occurred.

A great deal of effort has been expended over the past decade in identifying families with a history of breast cancer that can be accounted for by mutations in these two genes. Of course, small numbers of breast cancer cases in a single family might occur by chance, but chance alone cannot explain the many cases that occur in some families that do not carry *BRCA* mutations. These cases could be caused either by large deletions or loss-of-function mutations in *BRCA1* or *BRCA2* that are not detected by conventional screening techniques, or by mutations in other genes.

A few researchers have systematically attempted to find large deletions in the genomes of cancer families. These mutations would usually be missed by conventional sequencing or by the PTT. These studies have been aided by recent technical developments, such as MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION[22]. Genomic deletions in *BRCA1* are infrequent, accounting for only 5–10% of all germline mutations, and these mutations are probably even less common in *BRCA2* (REFS 23,24). Complex rearrangements that involve *BRCA1* — which often involve repetitive elements, such as *Alu* SEQUENCES — have been reported[25,26], but are also likely to be rare. If the proportion of mutations that are due to genomic deletions and rearrangements proves to be as high as 10% of the total, then these assays could become part of the complete mutational analysis of *BRCA1*.

Table 1 | **Techniques used to detect mutations in *BRCA1* and *BRCA2***

Technique	Advantages	Disadvantages
Protein-truncation test	Cheap; rapid; allows detection of genomic deletions	Mutations can be missed when gene product is very short; does not detect missense mutations; RNA required to examine small exons
Single-strand-conformation analysis of genomic DNA	Simple, well-established technique	Low sensitivity; labour-intensive; does not detect exon deletions*
Denaturing high-performance liquid chromatography	Detects almost all intra-exonic and splice-site mutations; rapid	Expensive equipment required; does not detect exon deletions*
DNA chips	Can potentially identify all sequence variants; very rapid	Expensive equipment required; high cost per chip
Direct sequencing	Identifies most intra-exonic and splice-site mutations	Expensive; exon deletions can be missed if detailed single-nucleotide-polymorphism analysis is not carried out
Multiplex ligation-dependent probe amplification	Detects all exon deletions	Cannot detect intra-exonic mutations

*This type of deletion is thought to be rare in most populations.

Since they were first reported in 1994, there have been difficulties in interpreting the effects of missense mutations on BRCA1 function. These mutations change a single amino acid, but otherwise leave the protein intact. In some cases, these are thought to disrupt protein function, but in other cases they are neutral polymorphic variants. More than 300 missense sequence variants in *BRCA1* have been submitted to the BIC database. It is challenging for geneticists to assess the risk of developing cancer for a woman who is found to carry an unclassified variant, and it is unsatisfactory for the patient to be given an ambiguous result. Factors that can be used to assess the pathogenicity of variants include the relative frequency of the variant in cases versus healthy controls and the co-segregation of the variant with disease in the family. There have been attempts to classify variants by measuring their effect on protein function *in vitro*[27–29], but there is currently no test that can be readily adapted for clinical purposes, and those that have been proposed have yet to be validated in large numbers of patients.

Founder effects

In the years immediately after the identification of *BRCA1*, several research teams undertook the categorization of mutations in different populations. In the first wave of number, Simard and colleagues identified recurrent mutations in a small series of families from Quebec, Canada[12]. Two mutations were seen more than once (185delAG and 5382insC), and families with these mutations were found to be of Jewish ancestry. Two years later, Offit and Neuhausen identified a third mutation (*BRCA2* 6174delT) that was also associated with Jewish ancestry[30]. Investigators have shown that if a Jewish woman does not carrry one of these three FOUNDER MUTATIONS it is highly unlikely that a different mutation will be found. Many researchers therefore believe that genetic testing in Jewish women could be limited to testing only for these three mutations[31,32].

Founder mutations have also been identified in Icelandic and Polish populations. As genetic testing is both cheap and reliable in these countries, population-based screening should be feasible[33,34]. Founder mutations also exist in other countries, but the number of additional variants of *BRCA* genes in these populations decreases the efficacy of screening. In some countries, it might be useful to identify common mutations before undertaking an extensive (and expensive) genomic search for cancer-causing mutations. However, in countries with ethnically mixed populations — such as the United Kingdom, Canada and the United States — the range of genetic variation is wide, so it has never been possible to narrow the search for cancer-causing genes, except in patients of Jewish ancestry. Because one of the three founder mutations is present in 2% of Ashkenazi Jews, this group has been studied extensively, and much of our knowledge about the penetrance, pathology and natural history of hereditary cancer has been derived from this relatively small group.

Penetrance

The penetrance of *BRCA* mutations is still a matter of intense research 10 years after the discovery of these genes (BOX 2). It is likely that more effort has gone into estimating the penetrance of *BRCA1* mutations than for mutations of any other gene. This investment is rational, given the high frequency of mutations and the obligation to communicate risk with accuracy prior to offering drastic preventive options, such as prophylactic mastectomy. It is perhaps disappointing that there is still controversy regarding which estimates of penetrance should be used to counsel women with *BRCA1* and *BRCA2* mutations; however, it is probably not surprising, as different studies continue to generate different figures. Penetrance — the lifetime risk of developing breast or ovarian cancer — is usually defined as the risk up to the age of 70 years . Both *BRCA1* and *BRCA2* mutations seem to have penetrance values for breast cancer of about 80%. It is widely accepted that the risk of ovarian cancer among carriers of *BRCA1* mutations (about 40%) exceeds that for carriers of *BRCA2* mutations (about 20%), and

MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION
A technique used to determine the copy number of multiple specific sequences in a single reaction. Two probes are hybridized to the target sequence and are joined together by ligation to make a copy of that sequence. The probes are designed so that all the products can be amplified using the same primer pair. The relative quantity of each product establishes the copy number of the target sequence.

ALU SEQUENCES
Short interspersed nuclear elements present at a high frequency in primate genomes. *Alu* sequences are amplified in the genome by retrotransposition. A complete *Alu* sequence is approximately 300 bp long and contains an A-rich region near the centre and a stretch of As at the 3′ end.

FOUNDER MUTATIONS
Specific mutations that appear repeatedly in ethnically defined groups because of a shared common ancestry and, typically, rapid population growth.

RAD51 FOCI
Discrete nuclear foci comprised of DNA-repair complexes that accumulate after endogenous or induced DNA damage. BRCA2 is a component of these foci and delivers RAD51 to the sites of DNA damage. BRCA1 might also be required to complete these foci.

that *BRCA2* carriers who develop ovarian cancer typically do so at an older age[35]. The risk of ovarian cancer is not the same for all *BRCA2* mutations; women with a mutation in the central part of the gene — the ovarian cancer cluster region (OCCR) — probably have a higher lifetime risk[36]. This is puzzling, given that these are all truncating mutations and that it was initially predicted that they would all have similar consequences for protein function. Conversly, male carriers of *BRCA2* mutations that lie outside the OCCR have been proposed to have an increased risk of developing prostate cancer[36,37].

The *BRCA2* mutation that is typically found in Jewish women (6174delT) is about as frequent in the general Jewish population as the 185delAG mutation[38], but is found less often than 185delAG in cases of breast cancer across all populations[39]. 6174delT is also found much less frequently in families with multiple cases[40]. The explanation for this discrepancy is that the penetrance of the 6174delT mutation is lower for breast cancer than that of 185delAG. Warner and colleagues estimated the breast cancer penetrance of the *BRCA2* 6174delT mutation to be low (28%, compared to 80% for *BRCA2* mutations in general). Again the basis for this difference is unknown, but it is interesting that this mutation lies in the OCCR.

It should also be remembered that estimates of penetrance vary between countries for reasons that are unrelated to genetics. For example, oral-contraceptive use, oophorectomy and parity all influence the risks of ovarian and breast cancer[41], and these factors vary between countries. Furthermore, as more women become aware that they are carriers of *BRCA* mutations, the penetrance values that are associated with each mutation will decrease, due to preventive efforts such as prophylactic oophorectomy and prophylactic mastectomy[8,9,42–44].

Functions of BRCA1 and BRCA2

Not all of the functions of the BRCA1 and BRCA2 proteins have been established, although many have been discovered during the past decade. BRCA2 is the larger of the two proteins and consists of 3,418 amino acids (FIG. 1). BRCA2 is involved in homologous recombination, but little else is known about its function. By contrast, several known functions of BRCA1 might underlie its role in carcinogenesis. These roles include DNA repair, cell-cycle-checkpoint control, protein ubiquitylation and chromatin remodelling (FIG. 2). These complex issues have been discussed in detail in several reviews[45–47].

DNA repair. Both BRCA1 and BRCA2 are implicated in the repair of DNA by homologous recombination (FIG. 2). BRCA1 associates with RAD51 in subnuclear clusters[48]. RAD51 is a key component of the mechanism in which DNA damage is repaired by homologous recombination. When DNA is damaged, both BRCA1 and RAD51 localize to to the damaged region, and BRCA1 is phosphorylated during this process. The nature of the interaction between RAD51 and BRCA1 is unknown, whereas it is known that BRCA2 can interact directly with RAD51, both through its BRC repeats and through a domain in its carboxyl terminus[49–51]. BRCA2 forms a complex with RAD51, holding it in an inactive state, and when BRCA2 is absent, RAD51 FOCI do not form after DNA damage.

Cells that are defective for BRCA1 or BRCA2 are hypersensitive to agents that crosslink DNA strands or that produce breaks in double-stranded DNA, such as cisplatin and mitomycin C[52–54]. In these cells, double-strand breaks are repaired by an error-prone mechanism — such as non-homologous end joining — and errors can lead to chromosomal rearrangements[55,56]. It is thought that the resulting chromosomal instability is a crucial feature of carcinogenesis. When cells are exposed to ionizing radiation, both BRCA1 and BRCA2 (together with RAD51) initiate homologous recombination and the repair of double-strand breaks[54]. Unsurprisingly, cells that express mutated BRCA1 and BRCA2 are hypersensitive to ionizing radiation and show error-prone repair. The levels of expression of BRCA1, BRCA2 and RAD51 increase in cells when they enter S phase, indicating that they function during or after DNA replication. So, BRCA1 and BRCA2 function in a common pathway that is responsible for the integrity of the genome and the maintenance of chromosomal stability[46].

As well as being involved in the repair of double-strand breaks, BRCA1 has also been implicated in nucleotide-excision repair. This involves two different mechanisms — transcription-coupled repair, in which the transcribed strand is preferentially repaired, and global genome repair, which does not show strand bias. BRCA1 might have a role in both transcription-coupled repair[57] and in global genome repair[58]. The effect of *BRCA1* deficiency on transcription-coupled repair might be limited to the blockage of the RNA polymerase II transcription machinery at the site of repair of 8-oxoguanine residues. This would be expected to be accompanied by specific patterns of somatic mutation (G>T transversions) in cancers that arise in carriers of *BRCA1* mutations. In the study that indicated a role for *BRCA1* in global genome repair[58], the effect of *BRCA1* deficiency was shown to be independent of p53 status, and no effect on transcription-coupled repair was seen. Unfortunately, progress in this area has been slowed by retractions of key publications, lack of replication studies and the use of different delivery and test systems.

Box 2 | **Estimating penetrance**

There is still controversy regarding which epidemiological method is best for estimating the cancer risk that is associated with a particular mutation. Scholars are in two camps — the first of these (which holds most of the original members of the Breast Cancer Linkage Consortium) hold that the risk of developing breast cancer is high for mutation carriers, and is largely independent of the ascertainment scheme by which the family was identified. This approach relies on the study of families that have a history of breast cancer as the proper and logical unit of study. Members of the other camp argue that penetrance estimates that are derived from studying large families will be too high and can generate unnecessary fear in the general population. They therefore promote population-based studies (the inclusion of women in a study regardless of their family history). Most genetic testing, however, is performed on women who ask to be tested, because of a family history of cancer. It is hoped that prospective studies will provide the most effective analysis yet, but these studies require large numbers of patients and extended periods of follow-up.

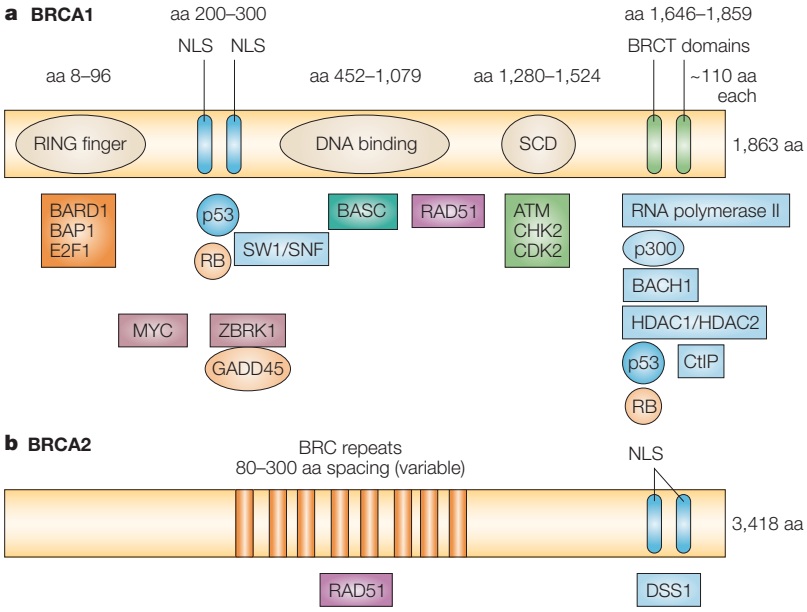

Figure 1 | **BRCA1 and BRCA2 functional domains, and selected binding partners.** **a** | BRCA1, which consists of 1,863 amino acids, contains several important functional domains that interact with a range of proteins. The RING-finger domain binds to BARD1 and this binding enhances the ubiquitin-ligase function of BRCA1. RING-finger binding to BAP1 and E2F1 has not been confirmed by *in vivo* studies. p53, MYC, RB and ZBRK1 all bind to a region of BRCA1 that includes the nuclear localization signals (NLSs). ZBRK1 is a zinc-finger protein that suppresses transcription through an interaction with GADD45. BRCA1 is required for this repression. SW1/SNF binding occurs between amino acids 260 and 553. The DNA-binding domain encompasses amino acids 452–1,079. It contributes to the DNA-repair-related functions of BRCA1, which are partly mediated through proteins that make up the BRCA1-associated surveillance complex (BASC). Several proteins (including MRE11, RAD50, NBS1, MDC1, ATM, CHK2 and CDK2) bind to the central region of BRCA1. SQ sequences (clusters of serine and threonine sequences), known as SQ-cluster domains (SCDs) are preferred sites of ATM phosphorylation. There are several SQ sequences between amino acids 1,280 and 1,524, whereas they are rare elsewhere in BRCA1. Two regions at the carboxyl terminus — known as the BRCT domains — are each about 110 amino acids long, and comprise amino acids 1,646–1,859. BRCT domains are found in many proteins involved in DNA-repair pathways, and bind to many proteins, including RNA polymerase II, p300, BACH1, histone deacetylases (HDACs) 1 and 2, p53, CtIP (carboxy-terminal-binding-protein interacting protein) and RB. The RNA polymerase II holoenzyme binds to both the amino and carboxyl termini of BRCA1; amino-terminal binding (not shown) is through the BARD1–BRCA1 complex. RAD51 and BRCA2 also bind BRCA1, and the three proteins colocalize in sub-nuclear foci. RAD51 binds BRCA1 directly, as shown. BRCA2 interacts with the BRCT domains; this interaction may be indirect, possibly as part of a complex with RAD51. **b** | BRCA2 is larger than BRCA1 (consisting of 3,418 amino acids), but only contains two known functional domains. The middle region of the protein contains eight BRC-repeat motifs, which are essential for its function in DNA repair and bind to the DNA recombinase RAD51. The DNA-repair activity of BRCA2 is regulated by DSS1, a small, acidic protein that seems to function as a necessary cofactor. DSS1 binds to the carboxy-terminal region of BRCA2, which also includes the NLSs. See REFS 46,47,178,179 for further details.

Checkpoint control. Another function of BRCA1 is in checkpoint control (FIG. 2). BRCA1 can exist as part of the BRCA1-associated genome-surveillance complex (BASC)[59]. This complex includes proteins such as Nijmegen breakage syndrome 1 (NBS1), the RAD50– MRE11 complex (which has exonuclease activity at double-strand breaks), ataxia telangiectasia mutated (ATM; which functions upstream of BRCA1 in the double-strand-break repair pathway), the MLH1–PMS1 and MSH2– MSH6 complexes, the BLM protein that is affected in Bloom syndrome, and DNA replication factor C.

The BRCT motif at the carboxyl terminus of BRCA1 (FIG. 1) is a common feature of proteins that are involved in DNA repair and/or cell-cycle checkpoints[60]. Interestingly, unlike BRCA1, the checkpoint function is preserved in BRCA2-deficient primary cells[55]. However, both *Brca1*$^{-/-}$ and *Brca2*$^{-/-}$ mice die during early stages of embryogenesis[61,62]. Loss of p53 or WAF1 (also known as p21) delays this embryonic lethality by a few days[63], indicating that the absence of checkpoint control might be a crucial step in tumorigenesis. Most *BRCA1*- and *BRCA2*-null cells undergo apoptosis because of intact checkpoint controls, but cells in which *BRCA1* or *BRCA2* are disrupted — as well as those in which key checkpoint proteins such as p53 or WAF1 are inactivated — survive in the presence of genomic instability. This results in the typically abnormal karyotypes that are seen in breast cancers associated with mutations in *BRCA1* and *BRCA2* (REFS 64–66). Despite the observation that most tumours from women with *BRCA1* or *BRCA2* mutations show loss of the corresponding wild-type allele, some cancers seem to arise in the presence of an intact wild-type allele. It has been proposed that in these cases — and possibly even in cases with two 'hits' that affect the same *BRCA* gene — the second event in tumorigenesis might involve the inactivation of a checkpoint gene, rather than loss of the second *BRCA1* or *BRCA2* allele[46,67].

Ubiquitylation. Ubiquitylation is the process by which proteins are tagged for degradation by the PROTEASOME. Many proteins that have ubiquitylation functions contain a RING-FINGER MOTIF. Both BRCA1 and its interacting protein BARD1 have a RING-finger motif near to their amino termini (FIG. 1), and it has been shown that the BRCA1–BARD1 complex functions in the ubiquitylation process[68] (FIG. 2). Disease-associated mutations in *BARD1*, however, are rare in *BRCA1*- and *BRCA2*-negative breast tumours[69–71]. Interesting recent studies indicate that BRCA1-mediated ubiquitylation occurs in response to replication stress[72], linking its ubiquitylation function to the DNA-damage response.

Chromatin remodelling. Chromatin remodelling occurs around double-strand DNA breaks and is thought to facilitate DNA repair. Several multimeric complexes — including BASC — are involved in this process, and BRCA1 seems to be a member not only of BASC, but also of a complex that contains the chromatin-remodelling proteins SW1 and SNF[73] (FIG. 2). There seems to be a direct interaction between BRCA1 and the SW1–SNF complex, indicating that these proteins function as a unit in the remodelling of chromatin that occurs around sites of DNA damage. Activation of other genes that are implicated in the response to DNA damage, such as *KU70* and *GADD45*, results from this interaction. Interestingly, mutations that affect another component of the SW1/SNF complex, SNF5, have been found in some patients with rare paediatric malignancies[74]. The fact that BRCA1 can also function as a histone deacetylase[75] and interacts with other proteins that are implicated in chromatin remodelling, such as

PROTEASOME
An organelle that breaks down proteins that have been targeted for degradation by ubiquitylation (by having a ubiquitin tag added to the protein). Lack of regulation of proteasomal degradation leads, for example, to loss of control of the cell cycle and seems to be an important step in tumorigenesis.

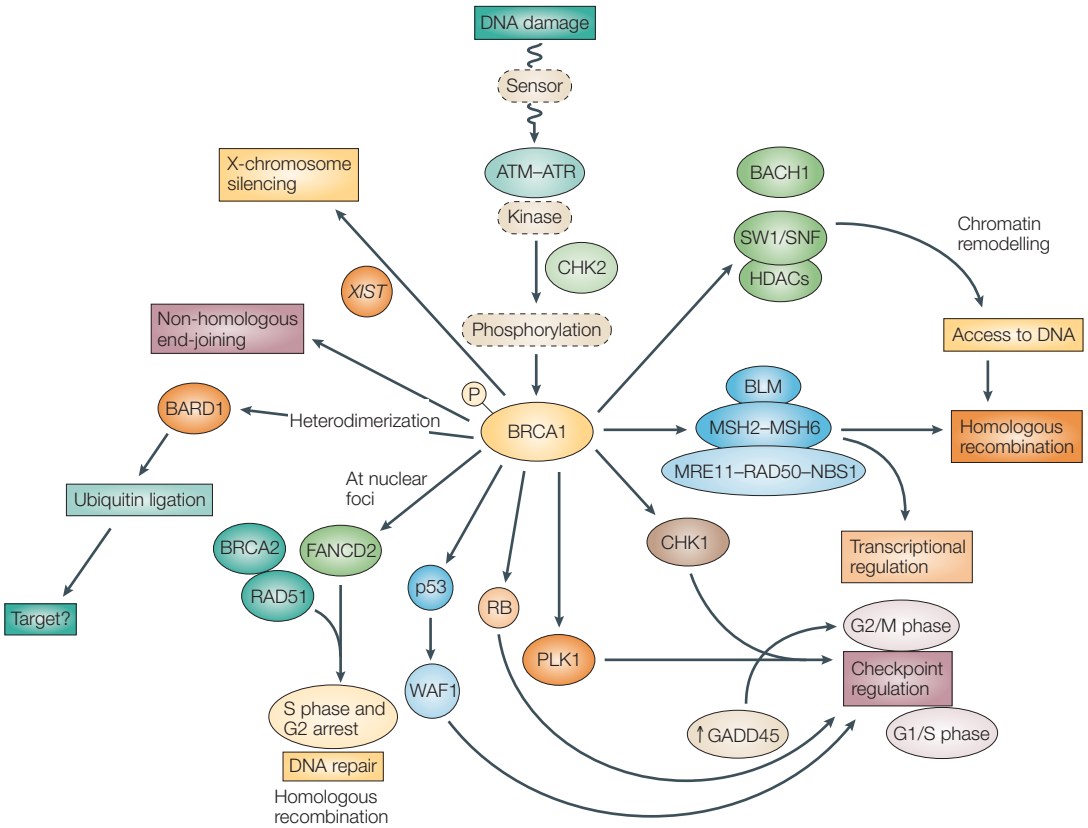

Figure 2 | **The BRCA1 network.** BRCA1 is an important component of pathways that regulate DNA repair, cell-cycle progression, ubiquitylation and transcriptional regulation. DNA damage (shown at the top of the figure) is thought to be one of the key triggers of BRCA1 activation. Several damage sensors, including ataxia telangiectasia mutated (ATM) and other kinases, are activated in response to DNA damage. CHK2 is also activated, and prevents cell division by phosphorylating BRCA1 and p53. Downstream targets of BRCA1 activation include p53 and the retinoblastoma protein (RB). BRCA2 and RAD51 form a complex that is believed to interact with FANCD2, which binds to BRCA1. This complex promotes S-phase or G2 arrest. BRCA1 forms a heterodimer with BARD1 to activate the ubiquitin-ligase function of BARD1, although its targets are unknown. DNA repair by homologous recombination is mediated by the BRCA1-associated surveillance complex (comprised of BLM, MSH2–MSH6 and MRE11–RAD50–NBS1). This complex also regulates transcription. BRCA1 has been shown to interact with X-inactive specific transcript (*XIST*) to mediate X-chromosome silencing, and also to mediate non-homologous end joining during DNA repair. BRCA1 can form complexes with both BACH1 and SW1/SNF to mediate chromatin remodelling and homologous recombination. HDACs regulate the access of the SW1/SNF–BRCA1 complex to DNA. Finally, BRCA1 interacts with CHK1 and polo-like kinase 1 (PLK1) to regulate the G2/M and G1/S checkpoints, possibly via GADD45; thereby linking BRCA1 to the regulation of apoptosis. See REFS 46,47,178,179 for further details.

BACH1 (REF. 76), emphasizes the importance of BRCA1 in processes that regulate DNA repair. Interestingly, a BRCA2-interacting protein, EMSY[77], also has DNA-repair functions, and mutations that affect this protein might underlie sporadic breast and ovarian cancers.

The Fanconi-anaemia connection

Fanconi anaemia is a rare recessive disease of childhood that features skeletal abnormalities, abnormal skin pigmentation, short stature and microphthalmia. Mutations in several genes can cause this condition, but all lead to chromosomal instability. When fibroblasts from children with Fanconi anaemia are exposed to mutagens such as mitomycin C or diepoxybutane they show an increased frequency of chromosome breaks compared with normal fibroblasts[78]. This is similar to the chromosomal instability that is seen in *Brca2*-deficient mice[55]. It was still surprising, however, when a rare form of Fanconi anaemia was shown to be caused by biallelic mutations in *BRCA2* (REF. 79). Other studies have shown that, in rare cases, children with medulloblastoma or Wilms' tumour also carry two truncating *BRCA2* mutations[80]. Homozygosity for *Brca1*-inactivating mutations, however, results in embryonic lethality, confirming the functional differences between the two proteins.

Models of tissue specificity

Why are the cancer phenotypes that are associated with *BRCA1* mutations so specific if the functions of the gene are so general? One possibility is that absence of BRCA1 could exacerbate the action of tissue-specific carcinogens, such as oestrogen. If breast cancer is the simple result of abnormal oestrogen sensitivity, however, then endometrial cancer should also be associated with *BRCA* mutations. Furthermore, oestrogen exposure has never been proven to be required for ovarian carcinogenesis. Monteiro postulated that tissue-specific differences in mitotic

RING-FINGER MOTIF
A motif comprised of cysteine and histidine residues interspaced with hydrophobic amino acids. Proteins that contain this motif usually have ubiquitin-ligase functions.

recombination might underlie the specificity of *BRCA* mutations for breast and ovarian cancers[81].

Elledge and Amon[82] proposed that only cells from breast and ovarian tissues are able to survive after acquiring defects in *BRCA1* because of the anti-apoptotic effects of oestrogen[85]. Anti-oestrogen therapies should therefore decrease the incidence of both breast and ovarian cancer in *BRCA1* carriers. Most *BRCA1*-related breast cancer cells, however, are oestrogen receptor (ER)-negative[83]. We propose a model that is a modification and an extension of that of Elledge and Amon, based on the assumption that the breast cancer stem cell is ER-negative, but the surrounding cells are ER-positive[84]. These surrounding cells might respond to oestrogen and send pro-survival signals to the ER-negative cancer stem cells[45].

Breast stem cells have a high intrinsic proliferative capacity. As a woman reaches menopause, oestrogen levels fall and there is a lower probablility that *BRCA1*-null cells will survive. Similarly, tamoxifen and oophorectomy are pro-apoptotic[86,87], so *BRCA1*-null cells that are exposed to either of these interventions are more likely to die early on. This could explain the sharp decrease in cancer risk that is seen at menopause in carriers of *BRCA1* mutations[88], which — notably — is not seen in carriers of *BRCA2* mutations. However, if *BRCA1*-null cells do escape this apoptotic mechanism, they would accumulate new mutations and begin to proliferate rapidly.

Is there a heterozygote phenotype?

There is no clear phenotype in murine or human carriers of heterozygous mutations in *BRCA1* or *BRCA2* — both species seem to develop normally in the presence of a single mutant allele. The possibility that a more subtle phenotype might exist in the breasts or ovaries of carriers has led several investigators to study tissues that are removed at the time of preventive or therapeutic surgery. Results have been conflicting[89–95], but overall there is scant evidence for an increase in the frequency of pre-malignant conditions in these organs compared to women from the general population. Several researchers have attempted to adapt the conventional model of cancer progression — which starts with hyperplasia, leading to *in situ* carcinoma and finally invasive cancer — to hereditary breast and ovarian cancers, but their findings have not been replicated[93,96,97].

Others researchers have studied *BRCA* heterozygosity in lymphocytes, rather than in healthy breast and ovarian cells[98–100]. There is some evidence that carriers of *BRCA1* mutations have an increased number of chromosome breaks, which is manifested as the presence of micronuclei in lymphocytes. However, it is not clear if these heterozygous cells are genetically unstable, and some of these results have been questioned[101,102].

Molecular carcinogenesis

In the early 1990s, it was anticipated that although germline *BRCA1* mutations are rare, a much greater proportion of breast cancers might be attributable to somatic mutations in *BRCA1*. This prediction was, of course, based on a paradigm introduced by Knudson in the 1970s — that the genes that are involved in hereditary cancer syndromes are the same as those that are involved in non-hereditary cancers, and that *BRCA1* would fall into the category of a classic tumour suppressor. This seemed to be strengthened by the observations of Devilee and colleagues that loss of heterozygosity for *BRCA1* occurs in cells from breast and ovarian tumours of patients with germline mutations[103], that the wild-type allele is 'lost' in *BRCA1*-linked breast cancer kindreds[104] and that cancer-associated *BRCA1* mutations result in loss of function. It was therefore surprising that alterations of single base pairs in the *BRCA1* coding region are only rarely associated with breast cancer[105,106] or ovarian cancer[107,108]. These findings seem to contradict the classic TWO-HIT MODEL OF TUMORIGENESIS.

Possible explanations for the small number of mutations that are seen include a narrow developmental window during which mutations can result in a recognizable phenotype[45,109]; an intrinsically low mutation rate for *BRCA1* (REF. 110); and the small number of mutation-prone breast stem cells[111]. It is important to note that the lack of somatic mutations that are associated with sporadic breast cancer does not mean that somatic mutations do not exist in patients with hereditary breast cancer — large-scale studies have not been published. If point mutations in *BRCA1* and *BRCA2* are rare or absent in all forms of breast cancer, this might represent an effect that is related to the spatiotemporal expression of *BRCA1* and *BRCA2* in the developing breast, because point mutations in other cancer-related genes — such as *TP53* — are common in breast cancer[112].

The transcription of *BRCA1* is in part regulated by the methylation of CpG islands at the 5′ end of the gene, and several studies have shown that altered methylation of this region can lead to gene silencing[113]. In breast cancers that are not associated with *BRCA1* mutations, methylation of this gene seems to be a frequent event[114,115]. Interestingly, breast tumours that are associated with *BRCA1* hypermethylation are histopathologically similar to those that are caused by inherited mutations in *BRCA1* (TABLE 2), in that they are high-grade, infiltrating ductal breast cancers that do not express ER. Low levels of *BRCA1* mRNA in unselected breast cancer specimens also support a role for the altered regulation of this gene in non-hereditary forms of breast cancer[116,117] and have been associated with a poor outcome following breast cancer in some studies[118], but not in others[119]. So, alterations in BRCA1 function might be more frequent that is commonly believed, as loss of function of BRCA1, whether by genetic or epigenetic mechanisms, tends to result in a recognizable phenotype. Further analysis of *BRCA1* methylation and expression patterns will be a challenge, as this analysis is laborious and requires well-preserved specimens.

Researchers have consistently been frustrated by the lack of a dependable antibody against BRCA1, which would allow them to evaluate protein levels in normal and tumour tissues. Several antibodies have been raised against various BRCA1 epitopes, but immunohistochemical analyses with these antibodies have not given reproducible results.

TWO-HIT MODEL OF TUMORIGENESIS
States that both alleles of a tumour-suppressor gene need to be inactivated to promote unregulated tumour-cell growth. A given allele could be inactivated due to inherited mutation (constitutional), somatic mutation or epigenetic silencing. Hereditary tumours would be caused by an inherited mutation and a somatic mutation; non-hereditary tumours would be the result of two somatic mutations.

Pathology

In 1993, the Breast Cancer Linkage Consortium (BCLC; see online links box) was created to facilitate collaborative breast cancer linkage studies. Admirably, the group stayed together following the cloning of the *BRCA* genes and has facilitated many clinical and pathological studies. Through the work of the BCLC and other groups, a clinicopathological phenotype for *BRCA1*-related breast cancer has emerged[120] (TABLE 2). Sobol and colleagues have suggested that these features are sufficiently specific that they can be used to identify probable carriers, based on tumour pathology[121]. *BRCA1*-related breast cancers are usually high-grade infiltrating ductal carcinomas. An atypical medullary phenotype (which is characterized by syncytial growth patterns, a smooth margin and abundant lymphocytic infiltration) is more common in *BRCA1*-related breast cancer than in matched controls[83,120] but occurs in only ~10% of *BRCA1*-related tumours. Conventional and molecular karyotyping studies have shown that the cells of these tumours are usually highly disorganized[64,65]. They are also usually ER-negative, particularly in younger women. Notably, the receptor tyrosine kinase ERBB2 (also known as HER2 or NEU) is overexpressed less often, compared with age-matched controls[122]. Many other immunohistochemical markers have been studied, most in small series.

Microarray analysis has allowed a more detailed analysis of the gene-expression patterns of various breast tumours. The most important initial categorization of breast cancers is into ER-positive and ER-negative subsets[123–125]. Most ER-positive cancers seem to show a luminal phenotype, as determined by expression of simple keratins, such as cytokeratins 8 and 18. By contrast, ER-negative cancers can be classified according to whether they overexpress ERBB2. Tumours that are both ER- and ERBB2-negative are characterized by the presence of basal cytokeratins, such as cytokeratins 5, 6 and 14 (REF. 123). It is perhaps unsurprising, therefore, that *BRCA1*-related breast cancers have a BASAL PHENOTYPE[126,127]. This is of some interest, because this phenotype is often associated with a specific expression pattern — apart from their ER- and ERBB2-negative status, tumours that mainly express cytokeratins 5 and 6, rather than cytokeratins 8 and 18, also tend to overexpress cyclin E and p53 and to underexpress KIP1 (also known as p27)[128]. All of these features have been associated with *BRCA1*-related breast cancer. It has been argued that breast cells that express only cytokeratins 5 and 6 are adult stem cells[129], and it is tempting to speculate that BRCA1 has some role in regulating the function of breast stem cells[111].

Another important question is whether breast cancers that are associated with *BRCA1* mutations behave more aggressively than sporadic tumours. Most of the evidence indicates that women with *BRCA1*-associated tumours have a worse outcome than women with sporadic breast cancers[130,131], but for those with mutations in *BRCA2* the situation is less clear[130]. The effect of treatment, however, has rarely been considered, and could be influenced by chemotherapy[132], as human *BRCA1*-null breast cancer cells are highly susceptible to this type of treatment. This effect is reversed when *BRCA1* is reintroduced[133]. Whether this information will be clinically useful is not yet known, but one small study found that carriers of *BRCA1* and *BRCA2* mutations are more likely to show a complete response to preoperative chemotherapy than non-carriers[134]. In general, progress has been hampered by the expense of mutation detection and the absence of large, well-characterized patient cohorts and appropriate comparison groups. However, such studies are now well underway in The Netherlands and Israel. The ability to assay for founder mutations in archived specimens and to link these to data about clinical outcomes allows large-scale, historical cohort studies to be carried out in these countries. A large prospective study is now underway in Poland.

The progression of *BRCA1*-associated breast tumours differs from that seen in sporadic cases in at least two ways. First, among *BRCA1*-mutation carriers, there is only a weak relation between the size of the primary cancer and the number of axillary lymph nodes to which the tumour spreads[135]. Several studies have reported, for example, that the poor prognosis that is associated with *BRCA1* mutations is restricted to women with node-negative disease[136–138]. Large *BRCA1*-related tumours are also much less likely to be node-positive than would be expected compared with sporadic tumours or tumours in women with *BRCA2* mutations. The reported poor prognosis for *BRCA1*-related node-negative breast cancer is surprising, as tumours that have not spread to the lymph nodes are usually associated with a significantly better outcome than are tumours of a similar size that are node-positive. It is possible that node-negative *BRCA1*-related breast cancers might show atypical metastatic routes of dissemination, posing challenges for breast cancer screening. The second atypical feature of *BRCA1*-associated cancers is their apparent ability to respond to oestrogen blockade, despite being ER-negative. Oophorectomy is associated with a reduction in the incidence of first and second primary breast cancers in *BRCA1* carriers[139,140], and tamoxifen is effective in preventing second primary cancers — most of which are ER-negative[140].

Identifying a phenotype that characterizes breast tumours that are associated with *BRCA2* mutations has been more difficult. In general, *BRCA2*-associated tumours cannot be readily distinguished from sporadic cancers on a morphological basis[120]. Overexpression of cyclin D1 seems to be a useful marker for *BRCA2*-related breast cancer[141], but further studies are required to confirm this. It is likely that significant differences do occur, if only because, like *BRCA1*-related tumours, they have specific genomic alterations — as indicated by their distinctive profiles obtained by comparative genomic hybridization[64].

Ovarian cancers that develop in both *BRCA1* and *BRCA2* carriers are usually serous papillary carcinomas, although endometrioid and clear-cell carcinomas also occur[142]. By contrast, mucinous and borderline ovarian carcinomas are rarely seen in carriers of mutations in either gene[142–144]. Primary cancers of the fallopian tube and peritoneum are also seen; these tumours also have a characteristic serous papillary appearance,

BASAL PHENOTYPE
Describes a relatively rare subtype of breast cancer that can be defined by immunohistochemistry. These tumours express markers that are typically seen in normal basal breast and skin epithelium, such as cytokeratins 5 and 6. This phenotype is often associated with a poor outcome.

Table 2 | **Key pathological characteristics of *BRCA1*- and *BRCA2*-associated breast cancers**

Phenotype	*BRCA1*-associated	*BRCA2*-associated
Morphology	Ductal, no specific type (75%); atypical medullary ~10%.	Ductal, no specific type (75%); atypical medullary < 5%; lobular or ductal with lobular features more prevalent than in women with *BRCA1* mutations (~10%)
Grade	High (grade 3; 75%)	Medium (grade 2; 45%) or high (grade 3; 45%)
Oestrogen-receptor expression	Negative (75%)	Positive (75%)
ERBB2 expression	Negative (95%)	Negative (95%)
p53 expression	Positive (50%)	Positive (40%)
Cyclin D1 expression	Negative (90%)	Positive (60%)
Carcinoma *in situ*	Rare	Common

indicating that these two genes restrict lineage choice in developing cancers. Microarray studies of ovarian carcinomas have shown that almost all cases have expression profiles that are similar to either *BRCA1*- or *BRCA2*-related cancers, indicating that abnormalities of one of the pathways involving these genes is essential for ovarian carcinogenesis[145].

Other breast cancer genes

The positional cloning of *BRCA1* was achieved less than four years after linkage was first reported in 1990, and it took little more than a year to identify *BRCA2* after its mapping in 1994. By 1996, it was clear that a substantial percentage of breast cancer families do not carry mutations in either of these two genes, indicating the probable existence of additional cancer-susceptibility genes. This led Mike Stratton and others to pursue the putative *BRCA3* gene. However, despite rapid advances in the high-throughput processing of DNA samples and the completion of a comprehensive genetic map in the 1990s and of the sequencing of the human genome in 2000, *BRCA3* remains elusive. If there is a *BRCA3*, it ought to have been found by now. Scientists such as Julian Peto have suggested that families with a history of breast cancer, but without *BRCA* mutations, might carry mutations that influence susceptibility in a more subtle or a more complicated manner, such as through gene–gene or gene–environment interactions[146]. Other researchers believe that no single gene influences cancer risk in these families. If so, then studies that are specific for particular ethinic groups (for example, Finns or French Canadians) might have a greater chance of success — many different genes might cause familial breast cancer clusters worldwide or within an ethnically mixed population, but only one or a few genes might contribute to cancer in an ethnically homogeneous population. Furthermore, a distinctive phenotype for a third class of inherited breast cancer has not emerged. It is important to note that the association between breast and ovarian cancer and the presence of male breast cancers were instrumental in the searches for *BRCA1* and *BRCA2*, respectively. It is also possible that the penetrance of *BRCA3* mutations is low.

Several reports have been published since 1995 that indicate the linkage of the breast cancer susceptibility phenotype to various regions of the genome[147–149], but none have been replicated in larger series[150,151]. As a result, enthusiasm for this form of gene identification seems to be waning; no genome-wide searches for *BRCA3* have been published. In Cambridge (United Kingdom) and Lyon (France), Mike Stratton and David Goldgar continue to assess random markers from a panel of 138 families in which three or more cases of breast cancer have been diagnosed. At a meeting of the BCLC in Madrid in 2003, they presented results from the first 400 markers. There was no compelling evidence of linkage to any region of the genome, so it is possible that no single gene underlies the breast cancer cases in these families. The supply of genetic markers is essentially unlimited, but families that are ideally suited for linkage studies are still hard to find, so alternative strategies have been proposed. In London, Ellen Solomon has collected data on several hundred pairs of sisters who have both developed breast cancer. Her model-free approach could be powerful if recessive genes underlie forms of inherited breast cancer that are not *BRCA1*- or *BRCA2*-associated, as has been indicated by some segregation analyses[152]. In addition, a study in Pakistan by Liede *et al.* reported that the parents of young women with breast cancer were more likely to be in consanguineous marriages[153].

If it is not possible to define a characteristic *BRCA3*-type family, then it might be possible to identify a *BRCA3*-associated cancer fingerprint. Conventional histopathology[154], microarray technology, loss of heterozygosity and comparative genome-hybridization arrays[155] have all been used to try to identify a tumour-specific, *BRCA3*-associated signature. These studies support the idea that familial breast cancers that are not associated with mutations in *BRCA1* or *BRCA2* are probably heterogeneous although, overall, they are less aggressive than non-familial breast cancer. It is possible that Peto is right, and that no other highly penetrant alleles that predispose to breast cancer exist in ethnically mixed western populations. In this case, the clustering of breast cancer in families is probably the result of a mixture of many interacting genes and chance. Paul Pharoah and his colleagues[156] have proposed a model in which risk is not symmetrically distributed — the 50% of the population that is at the highest risk would account for almost 90% of affected individuals.

It was initially proposed by Swift in the early 1970s that the first-degree relatives of children with ataxia telangiectasia, which is caused by mutations in *ATM*, have

an increased risk of developing breast cancer[157]. In the past decade, this hypothesis has been confirmed by more detailed epidemiological studies[158] and by surveys of specific *ATM* mutations in unselected breast cancer cases[159]. By contrast, a study of the role of *ATM* mutations in breast cancer reported no relation between the two[160]. These investigators, however, screened only for mutations in their population using the PTT. It is possible that truncating mutations are not pathogenic, but that other types of mutation — for example, missense mutations — are pathogenic[161,162]. One such mutation, *ATM**7271T>G, which probably originated in the Orkney islands (which are located north of Scotland), has been associated with a high risk of breast cancer in a few families[159,163], but it is not frequently associated with hereditary breast cancer[164]. A more general role for missense mutations could be explained by a dominant-negative effect, in which the mutation might confer functions that are not present in the wild-type protein. By contrast, when a truncating mutation is present on one *ATM* allele but the other is normal, the product of the normal allele might be sufficient to maintain normal function[162]. A large-scale study is therefore required to identify ATM mutations in patients with breast cancer.

The biggest discovery in the field of familial breast cancer research since the discovery of *BRCA2* was probably the identification of the *CHK2* gene. The *CHK2* founder mutation (1100delC), which abolishes the kinase activity of the gene product[165], was found to confer a moderately increased risk of breast cancer in Europeans[166]. There is a large degree of regional variation in the prevalence of this allele, but it seems to be most common in northern Europe (partcularly in The Netherlands and Finland), and is rare among North Americans[167–169]. *CHK2* encodes the human homologue of the yeast checkpoint kinases Cds1 and Rad53 (REFS 170,171). CHK2 is activated in response to DNA damage that is induced by ionizing radiation[172–174], and activation of this protein prevents cells from entering mitosis. Activated CHK2 phosphorylates BRCA1 and p53, thereby regulating their functions[175–177].

Future directions

Despite the results of the studies described above, mutations in *ATM* and *CHK2* are not sufficiently prevalent or penetrant to make these genes candidates for a third breast cancer gene — mutations in these genes only account for a small percentage of the breast cancer cases that occur in families that do not carry *BRCA1* or *BRCA2* mutations, and few clinicians offer tests for mutations in these genes. During the past decade, it has become apparent that only two genes are clearly associated with inherited forms of breast cancer, and that no simple Mendelian model will explain the remaining familial clusters. At the same time, we have come to accept that genetic testing for *BRCA1* and *BRCA2* has an important place in medical practice. We have also learned that defective DNA repair is a common characteristic of all of the genes that underlie the hereditary breast cancer syndromes. It will be important to determine the reason that the tumorigenic effects of these mutations predominantly target breast and ovarian cells and the reason that different mutations in the same gene confer different levels of risk for the same type of cancer.

In future studies, it will be important to determine the roles of ER-negative breast stem cells in tumorigenesis. Further molecular characterization of *BRCA1*- and *BRCA2*-associated breast tumours should also bring improvements in selecting effective chemotherapy regimens and predicting prognosis. Finally, knowledge of the specific molecular-genetic signatures of *BRCA1*- and *BRCA2*-associated breast and ovarian cancers might foster the development of new targeted therapies.

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Competing interests statement

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