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Germ line mutations associated with breast cancer susceptibility

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Abstract

Laboratory-based research in germ line mutations associated with breast cancer susceptibility is rapidly being integrated into clinical practice with profound implications. A Medline search was performed for all relevant articles published since 1990. Where appropriate, historical articles referenced in those identified were also reviewed. The results suggested that while mutations in the *BRCA1* and *BRCA2* genes are the most clinically relevant, much of the data on which clinical decisions are based must be interpreted with wide confidence intervals. Between 1 in 152 and 1 in 833 individuals carry such mutations. They account for less than 5% of all breast cancer, but up to 10% of cancers in those under the age of 40 years. Founder mutations are responsible for a larger proportion of breast cancer cases within certain inbred communities. Phenotypic expression and penetrance of different mutations is not currently predictable and estimates of penetrance are largely based on highly selected populations. *BRCA1* mutations are more commonly associated with ovarian cancer than *BRCA2* mutations. *BRCA1* cancers tend to have more distinct pathological features and are usually oestrogen receptor (ER)-negative. To conclude, the evidence in this review suggests that caution should be exercised when translating scientific progress in breast cancer germ line genetics into clinical practice. Most of the available data are derived from studies on highly selected populations. The importance of other less penetrant, but more prevalent, germ line mutations may be realised in the future.

Keywords: Breast; Cancer; Germ line; Mutations; Genetic; Genes

1. Introduction

The inception of a cancer occurs in cell division with a chance mutation. Thus cancer incidence depends on the number of cells at risk, their rate of division, the frequency of cancerous mutations and the viability of mutated cells. Cancerous transformation of a cell is therefore a rare event, occurring for breast cancer somewhere in the order of once in every few million cell divisions. This rate may be increased by environmental stimuli (e.g. irradiation) and after initial mutation a cancer cell may undergo many subsequent genetic alterations. Hence in sporadic breast cancer, mutations are commonly found in tumour cells. Such somatic mutations may determine the phenotype of a particular breast cancer and may be of clinical value in determining prognosis. However, only germ line (inherited) mutations can predetermine an individual's risk of developing breast cancer.

The significance of a germ line mutation depends upon its prevalence and its penetrance. If either of these factors is high then the mutation is likely to be clinically important. Highly penetrant mutations that are also prevalent are, of course likely to be relatively easy to identify because of the clustering of cases in families. Mutations of low penetrance may be more prevalent and may account for a higher percentage of all breast cancers, but identifying carriers may be difficult. It is likely that there are few highly penetrant mutations that cause breast cancer (*BRCA1* and *BRCA*2). There may be more low penetrance mutations (ataxia telangiectasia mutant (*ATM*) and others yet to be identified). There are also a few rare mutations, which produce recognisable multicancer syndromes (*TP53*, *PTEN*, *BLM*).

In this review, we provide an overview of the germ line genetic mutations related to increased breast cancer risk, with particular emphasis on the hereditary breast–ovarian cancer syndrome.

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2. The background

Population-based studies have attempted to define the cancer risk associated with a positive family history of breast cancer. One of the largest was conducted in Sweden using mailed questionnaires which were supported by pathology and hospital reports [1]. This involved 1330 women with histologically confirmed newly diagnosed breast cancer within a defined geographical region and included age-matched controls without breast cancer. Breast cancer in a first-degree relative was found in 11.2% of breast cancer patients as opposed to 6.7% of controls ($P < 0.01$), yielding a standardised relative risk (RR) of 1.7. A similar magnitude of relative risk was obtained from population-based studies in Canada and in the United States Nurses' Health Study. The Canadian study [2] consisted of 577 female breast cancer patients and 826 controls in a limited geographical area in Southern Alberta. The age-adjusted relative risks were 2.2–2.3 (95% confidence interval (CI) 1.3–3.8) for women with a mother or a sister with breast cancer. The Nurses' Health Study [3] consisted of 1159 nurses who had breast cancer and 11 590 controls. Both groups were sent mailed questionnaires requesting health-related information and family history of breast cancer in a sister, mother or both. A maternal history of cancer conferred a relative risk of 1.8 (95% CI 1.5–2.3), and a positive sister history a relative risk of 2.5 (95% CI 1.9–3.3). Examination of these relative risks with stratification of possibly confounding non-heritable components such as use of oral contraceptives, other hormone use and geographical locale showed no substantial differences across the strata.

Anderson and colleagues first reported heterogeneity of risk among breast cancer families in the early 1970s [4–6]. These studies challenged the notion that familial breast cancer risk was homogeneous and suggested that rare, higher-risk families with specific clinical and genetic factors may be obscured by previous large population-based studies. To identify these families, Anderson assembled a study cohort consisting of 234 breast cancer patients with a family history of the disease in two or more first- or second-degree relatives. Results showed a significant correlation between familiarity and early onset (premenopausal) and bilateral disease, with each conferring a 3- and 5-fold increase in risk among relatives, respectively. Significantly, analysis identified a group of women whose sisters and mothers both had breast cancer and who had a 50-fold risk of the disease compared with controls. Comparisons of the pedigrees provided no evidence of a difference between paternal and maternal transmission, suggesting that males are equally involved in the transmission of breast cancer susceptibility. In 1972, Lynch and colleagues [7] analysed a cohort of 34 families each having two or more first-degree relatives with breast cancer. In one family, there were eight histologically proven breast cancers through four generations. As 3 out of 6 women in a single generation developed the disease, the trait was suggested to be due to transmission of a single dominant gene.

In 1984, Williams and colleagues [8] provided evidence of an autosomal dominant breast cancer susceptibility gene with an age-related prevalence based on a study of 300 Danish breast cancer patients from 200 pedigrees. Using complex segregation analysis to test different models of genetic inheritance, a dominant locus of low frequency was found to give the best fit to the distribution of disease. Penetrance of the abnormal allele was also found to increase with age, and by age 80 years a female heterozygote was estimated to have a 57% risk of developing breast cancer. These findings were supported in 1988 by Newman and associates [9], who carried out complex segregation analysis for 1579 families of consecutive breast cancer patients diagnosed before the age of 55 years. Family history of breast cancer and other cancers at any age was confirmed in the mothers and sisters. An autosomal dominant model and a highly penetrant susceptibility allele fully explained the clustering of cases within families and the frequency of this allele was estimated at 0.0006, with a lifetime risk of breast cancer among carriers of 82%. These findings were supported by another segregation analysis in a large study by Claus and associates [10]. Data were obtained from the Cancer and Steroid Hormone (CASH) study, a multi-centre population-based case–control study. A total of 4730 histologically confirmed breast cancer patients aged between 20 and 54 years were identified and matched with 4688 controls for geographical region and 5-year categories of age. The number of affected first-degree relatives and their age at diagnosis were the most important risk factors for breast cancer, with a sharp increase in risk for women with at least two affected first-degree relatives. Again, analysis found that an autosomal dominant model provided the best fit to the data, with a population frequency of 0.0033.

The search for the putative breast cancer gene employed the technique of genetic linkage. Essentially, linkage refers to the fact that if two or more genetic loci lie in very close physical proximity, they are likely to segregate together during the process of meiosis. The usual statistical measure of linkage is the lod score, which is the 'logarithm of the odds'. This is the $\log_{10}$ of the odds in favour of finding the observed combination of alleles at the loci studied if they are linked. A lod score of $+3$ or greater is considered to be strong evidence of linkage (1000:1 odds for linkage). For the purpose of gene mapping, linkage analysis uses known polymorphic markers. These are short polymorphic tandem-repeats scattered throughout the genome which

are easily amplified by the polymerase chain reaction (PCR). The segregation of disease phenotypes relative to these polymorphic markers can then be analysed. These initial investigations to map the site of the breast cancer susceptibility gene therefore required recruitment of large families with multiple affected relatives.

In 1990, understanding of genes involved in breast cancer susceptibility was significantly advanced by the landmark report of Hall and colleagues that linked families with early-onset breast cancer to chromosome 17q12 [11]. The study population consisted of 23 extended families with 146 breast cancer cases selected for young age at diagnosis, bilateral disease or male breast cancer. Using four polymorphic markers at chromosome 17q12, disease was found to link within a recombination distance of 0.014 of the *D17S74* marker in 40% of the families and specifically those with early-onset disease. The following year, using the same marker in a study population of five families each with at least 5 cases of histologically confirmed breast cancer and 2 cases of ovarian cancer, Narod and colleagues [12] showed that three of these families were found to have positive linkage, implying a link between the same breast cancer susceptibility gene and the hereditary breast–ovarian syndrome. In 1993, Feunteun and colleagues [13] confirmed the presence of a breast cancer susceptibility gene in hereditary breast-specific and breast-ovarian families. Using a study population of families with at least 4 cases of breast or ovarian cancer, four polymorphic markers spanning a region of approximately 15cM on chromosome 17q12 were used to type 370 individuals. The presence of a large family with 28 affected members and a high probability of linkage allowed the identification of recombinant events in affected individual relatives, which narrowed the locality of the breast cancer susceptibility gene to within a 6cM interval. In 1994, by developing a transcriptional map of this 600 kb region, Miki and colleagues [14] found a single transcription unit where mutations were found to segregate to kindreds with 17q-linked susceptibility for breast and ovarian cancers. Thus, *BRCA1* was cloned. The large size and complexity of the gene was realised from the outset as *BRCA1* was found to be composed of 22 coding exons distributed over 100 kb of genomic DNA.

As only 45% of families with multiple cases of early-onset breast cancer showed evidence of linkage to *BRCA1* and initial studies had shown no apparent association between *BRCA1* and male breast cancer, the search for a second major breast cancer susceptibility gene continued. In 1994, Wooster and associates [15] performed a genetic linkage search using 15 families that had multiple cases of early-onset breast cancer, but no evidence of linkage to *BRCA1*. The search for *BRCA2* was focused on chromosome 13q. Haplotype analysis confirmed the cosegregation of disease with chromosome 13q markers and recombination between different closely spaced markers isolated the location of the putative *BRCA2* gene. The precise localisation of *BRCA2* was unexpectedly assisted by the discovery of a homozygous deletion in a pancreatic carcinoma that suggested the presence of a tumour suppressor gene [16] in this area. This deletion was localised to a 1cM region at chromosome 13q12.3, called the DPC (deletion in a pancreatic carcinoma). The DPC is encompassed entirely by the 6cM region of the putative *BRCA2* gene. Gene mapping of this area in 46 early-onset breast cancer families that had shown previous linkage to *BRCA2*, but not *BRCA1*, led to identification of the *BRCA2* gene [17]. Since these genes were cloned, this has become a fast developing field with at least three or four articles appearing in high-ranking journals every month. This review therefore represents a snapshot in the evolution of breast cancer genetics.

3. Prevalence of *BRCA1* and *BRCA2* mutations in hereditary breast cancer families

The *BRCA1* and *BRCA2* genes are thought to account for the large majority of hereditary breast–ovarian cancer families [18,19]. Much of the earlier work estimating the prevalence and penetrance of these two genes required collation of shared databases of large families with multiple cases of breast and/or ovarian cancer for the purpose of linkage analysis. Many of these studies were carried out by the Breast Cancer Linkage Consortium (BCLC), an international network of scientists founded in 1989 in Lyon, France, which now has genetic data for over 700 families from Europe, Canada and the USA. Definitions of what constitutes a hereditary breast and ovarian cancer (HBOC) family has varied, but a working definition was generally taken as families with 4 or more cases of early-onset breast (age < 60 years) or ovarian cancer, with at least 1 case of ovarian cancer.

In a linkage analysis of 145 such families gathered by the BCLC using 11 markers flanking the *BRCA1* gene, Narod and colleagues [18] found that none of the 13 families with male breast cancer showed evidence of linkage to *BRCA1*. When families with male breast cancer were excluded, 92% (95% CI 76–100%) of families with 2 or more cases of ovarian cancer showed evidence of linkage to *BRCA1*. Only 10 families without male breast cancer were considered unlikely to be linked to the *BRCA1* locus. Of these, seven were later found to show positive linkage to *BRCA2* and the remaining three families were in fact found to carry *BRCA1* mutations when mutation analysis of the gene became available (the misleading results were due to early-onset sporadic breast cancer in these *BRCA1* families) [19].

Table 1
Prevalence of *BRCA1*/*BRCA2* mutations

Estimated frequency of mutations (95% CI)	Estimated carrier prevalence in population (95% CI)	Study design
0.0014 (0.002–0.011)	1/345 (1/2596–1/46)	Families of US ovarian cancer cases and controls [23]
0.003 (...)	1/152 (...)	Families of US breast cancer cases and controls [10]
0.0006 (0.002–0.001)	1/833 (1/2500–1/500)	Families of breast or ovarian cancer cases in England and Wales [22]
0.0012 (. . .)		Early onset breast cancer in UK [24]

CI, confidence interval.

The contribution of *BRCA1* to the majority of HBOC families had been suggested earlier by Easton and colleagues [20] in a collaborative linkage study involving 214 breast cancer families, including 57 breast–ovarian families. This linkage analysis had estimated that 90% of breast–ovarian cancer families and 45% of site-specific breast cancer families were linked to *BRCA1*. A recent review [21] of the relative contribution of *BRCA1* and *BRCA2* to HBOC families showed that for 237 families with at least four cases of breast cancer (regardless of ovarian and other cancers), linkage to *BRCA1*, *BRCA2* and to neither gene was estimated at 63, 32 and 16% respectively. This suggests that more breast cancer susceptibility genes exist. In HBOC families, most were linked to *BRCA1* (81%) and *BRCA2* (14%). However, in families with 4 or 5 cases of breast cancer (and no ovarian cancer), 67% were not linked to either *BRCA1* or *BRCA2*.

4. Prevalence of *BRCA1* and *BRCA2* mutations in the general population

Due to the limitations of current mutation detection techniques for large genes with extensive allelic heterogeneity such as *BRCA1* and *BRCA2*, it is not yet feasible to analyse large samples of the population for all possible mutations and hence determine the population prevalence of mutations in these genes. However, using population-based case–controlled studies, highly penetrant autosomal dominant breast cancer susceptibility genes such as these are thought to be rare, with the exception of some distinct population groups. The range of estimates obtained between the different studies (Table 1) may be partially explained by differences in the case-control designs. Estimates by Ford and colleagues (1/833) [22] and Whittemore and associates (1/345) [23] were based on families that contained both breast and ovarian cancers among first-degree relatives. This probably segregates for *BRCA1* with a minor contribution from *BRCA2* and other susceptibility genes. The Claus estimate [10] described earlier was based on a case–control study of histologically confirmed breast cancers with few cases of ovarian cancer and is likely to segregate for other breast cancer susceptibility genes as well as *BRCA1*.

From their case–controlled studies, Ford and colleagues [22] and Whittemore and associates [23] attempted to estimate the age-specific proportion of breast and ovarian cancers that arise from *BRCA1* mutations (Table 2). While variations in their estimates may be due to differences in the study population, both studies concur that *BRCA1* mutations are responsible for only a small minority of all breast cancers, but the proportion due to *BRCA1* is greater in young women.

Recently, Peto and colleagues [24] reported a prevalence study of *BRCA1* and *BRCA2*. Mutation analysis was performed on blood samples obtained from 617 participants in the UK National Case Control Study Group. This consists of two groups of women with breast cancer, one group diagnosed before the age of 36 years and one group diagnosed between 36 and 45 years. Deleterious mutations in *BRCA1* were detected in 3.1% of women diagnosed before 36 years and 1.9% of women diagnosed between 36 and 45 years. Using previous penetrance estimates, the prevalence of *BRCA1* mutations in the general population was calculated to be 0.0011, closely mirroring the previous estimates by Ford and Whittemore. The prevalence of BRCA2 in this study was similar to that of *BRCA1*, with 2.4% of the under 36 year age group and 2.2% of the 36 to 45 year age group found to have deleterious mutations at the *BRCA2* locus.

Table 2
Proportion of cases due to *BRCA1*

Age at diagnosis (years)	Proportion of cases due to *BRCA1*			
	Breast cancer (%)		Ovarian cancer (%)	
	Ford [22]	Whittemore [23]	Ford [22]	Whittemore [23]
20–29	7.5	11.2	5.9	17.9
30–39	5.1	10.7	5.6	17.5
40–49	2.2	8.6	4.6	6.8
50–59	1.4	5.8	2.6	6.4
60–69	0.8	0.7	1.8	3.1
70–79		0.6		2.8
15–69	1.7	4.2	2.8	5.3
15–79		3.0		4.4

5. Penetrance of *BRCA1*

Much of the early work in this area was carried out by the BCLC. Summarising their early experience, Easton and colleagues [25] estimated the cumulative risk of breast and ovarian cancer based on the incidence of these cancers in 33 families with at least 4 cases in total of either ovarian cancer diagnosed at any age or of breast cancer diagnosed below the age of 60 years. The incidence of breast cancer was 85% and the incidence of either breast or ovarian cancer 95% by age 70 years. In a recent penetrance analysis by the BCLC reported by Ford and associates [21], the study population consisted of 237 families with at least 4 cases of breast cancer unselected for ovarian cancer and included the cases reported earlier by Easton. Penetrance estimates in this larger population were very similar to the earlier study (Table 3).

Although these studies suggest that more than half of *BRCA1* carriers will be affected with either breast or ovarian cancer by the age of 50 years, these risks may not be representative of the full spectrum of *BRCA1* mutations due to the selection of very high-risk families. Studies which overcome this bias have been carried out in population groups where founder mutations have facilitated site-specific mutation screening of large numbers of subjects (see Section 9). Struewing and colleagues [26] analysed the risk of cancer in 120 carriers of the 185delAG, 5382insC (*BRCA1*) and 6174delT (*BRCA2*) mutations. They were identified among 5318 volunteer Jewish subjects in the Baltimore area not selected for family history. The risk of breast cancer was found to be 33% (95% CI 23–55%) by the age of 50 years, with no significant difference in risk between different mutations. The ovarian cancer risk was 7% (95% CI 2–14%) by the age 50 years and 16% (95% CI 6–28%) by the age of 70 years, much lower than the BCLC estimates. A similar study was conducted among 268 histologically proven breast cancer patients of Ashkenazi Jewish descent in the New York area by Fodor and associates [27]. While 42% of the study subjects had relatives with breast cancer, only 5 had three or more affected relatives and the majority of women were therefore considered to be at low or moderate risk for breast cancer based on their family history. For the *BRCA1* 185delAG and *BRCA2* 6174delT mutations, the lifetime risk for breast cancer was calculated to be 36%, similar to the Baltimore data. Similar results were obtained by Dorum and colleagues [28], who examined the penetrance of the Norwegian *BRCA1* 1675delA and 1135insA founder mutations, and Hopper and associates [29] in a study of protein truncating mutations in Australia. Both these series consisted of probands with a modest breast and/ovarian cancer family history, and demonstrate significantly lower penetrance estimates than the BCLC. Even in high-risk families, variable penetrance of a mutation can be observed. In a 4184delTCAA mutation (*BRCA1*) family, Friedman and colleagues [30] reported that two carriers developed bilateral and unilateral breast cancers by the age of 46 and 49 years, respectively, another developed breast cancer at the age of 78 years, and two other carriers remain cancer-free at age 73 and 81 years. These variations in cancer phenotype and the degree of familial clustering in carriers of the same mutation suggest the presence of presently unknown modifying factors, either environmental or (more likely) genetic, that alter the clinical expression of these mutations. The penetrance estimates from these studies can only therefore be taken as averages, incorporating some mutation carriers that are at very high risk and others with only moderately elevated risk.

Several authors have attempted to explain the difference in penetrance estimates between these later studies and earlier, large pedigree-based reports [31,32]. Essentially, these variations may be methodological (with pedigree-based studies by definition having large families with large numbers of affected relatives), biological (due to modifying genes within these large families), stochastic (due to chance distribution of cases within the populations studied) or environmental (diet, smoking or other modifying lifestyle) factors. Furthermore, while the linkage studies reviewed here have full histological confirmation of cancers in multiple affected relatives, most studies based on general population groups have relied on interview of the index patient for the ascertainment of the cancer status of their relatives. General population studies have also been largely based on

Table 3
Cumulated risks of breast and ovarian cancer in *BRCA1* mutation carriers

	Easton [25]			Ford D [21] (95% CI)	
Age (years)	Breast cancer	Ovarian cancer	Either cancer	Breast cancer	Either cancer
30	0.032	0.0017	0.034	0.036 (0–0.14)	0.36 (0–0.14)
40	0.191	0.0061	0.195	0.18 (0–0.36)	0.18 (0–0.36)
50	0.508	0.227	0.619	0.49 (0.28–0.64)	0.57 (0.33–0.73)
60	0.542	0.298	0.678	0.64 (0.43–0.77)	0.75 (0.53–0.87)
70	0.850	0.633	0.945	0.71 (0.53–0.82)	0.83 (0.65–0.92)

CI, confidence interval.

determining the carrier status in relation to definite founder mutations within that population. The BCLC studies have used a lod score method, which is independent of the type of mutation and is more sensitive than most mutation analyses carried out in general population screening.

6. Penetrance of *BRCA2*

Less is known about the penetrance of *BRCA2*. Estimates by Easton and associates [33] were based on the incidence of cancers in *BRCA2* mutation carriers from two large families that had shown linkage to the *BRCA2* linkage at chromosome 13q12. Pedigrees included all second-degree relatives of breast cancer patients diagnosed before the age of 60 years and male breast cancers of any age. A total of 41 female and 4 male breast cancers were studied. Breast cancer risk for women was found to be similar to that of *BRCA1* mutation carriers, but ovarian cancer risk appeared to be lower. In a recent review of *BRCA1* and *BRCA2* penetrance by the BCLC [21], penetrance of *BRCA2* was estimated based on 32 *BRCA2* families that were typed with genetic markers flanking the gene and these estimates were comparable with those reported by Easton (Table 4). Compared with *BRCA1* penetrance, the *BRCA2* rates are slightly lower for younger age groups, but are not significantly different at any age. The risk for ovarian cancer appears to be lower for *BRCA2* than *BRCA1* carriers.

7. Methods of mutation screening

Different mutation detection techniques have evolved which provide a suitable procedure for virtually any experimental situation [34]. Essentially, the choice of which mutation analysis to use is a compromise between two ideals. Methods such as direct sequencing and chemical mismatch cleavage (CMC) detect nearly 100% of small mutations, but have the disadvantages of being very time consuming (sequencing) or involving the use of toxic chemicals (CMC). Although use of fluorescent primers and automated sequencers have improved their throughput and avoided the need for toxic chemicals [35], these methods are generally chosen in settings where there is a limited study population size and where the maximum detection rate is required. Other methods such as single-stranded conformational polymorphism (SSCP) or heteroduplex analysis (HDA), while having a worse detection rate for small deletions (70–100%), have the advantage of being easier to run and are more suitable for higher throughput research applications [36]. These methods rely on enzymatic amplification of defined DNA segments and along with the protein truncation test (PTT) are the techniques currently employed for *BRCA1* and *BRCA2* mutation screening by most gene testing laboratories [37]. Although rapid and relatively robust, they are unable to fully characterise the nature of changes in the gene that are identified. Full characterisation still requires direct sequencing of the affected segment, which remains the gold standard for mutation analysis. Previously expensive and extremely labour-intensive, automation with fluorescent detection technologies has made direct sequencing more efficient and it is likely to become the primary method of mutation detection in the near future. However, as we shall show, some significant mutations will be missed by direct sequencing.

The SSCP assay was first reported by Orita and colleagues in 1989 [36] and has been one of the most widely used methods of analysis for mutations in *BRCA1* and *BRCA2*. Following PCR amplification, DNA fragments are denatured and electrophoresed through a non-denaturing polyacrylamide gel, migration being determined by secondary structure, which is in turn determined by base composition. The sensitivity of SSCP is dependent on the size of the amplified fragment and is estimated to be between 70 and 95% in PCR products of 200 base pairs or less [38], decreasing to 50% when fragments larger than 400 base pairs are analysed.

HDA relies on the formation of heteroduplexes between wild-type and mutant DNA strands during the late cycles of the PCR. Heteroduplexes are thought to migrate more slowly than their corresponding homoplexes due to the more 'open' double-stranded conformation of the mismatched bases, with single base

Table 4
Cumulative risks of breast and ovarian cancer in *BRCA2* carriers

Age(years)	Easton [25]		Breast Cancer Linkage Consortium [21] (95% CI)	
	Female breast cancer	Male breast cancer	Female breast cancer	Ovarian cancer
30	0.013	–	0.006 (0–0.19)	0.00
40	0.13	0.0008	0.12 (0–0.24)	0.00
50	0.60	0.008	0.28 (0.090–0.44)	0.004 (0–0.011)
60	0.71	0.029	0.48 (0.22–0.66)	0.075 (0–0.15)
70	0.80	0.063	0.84 (0.43–0.95)	0.27 (0–0.47)

CI, confidence interval.

deletions being more easily detected than single base substitutions due to a larger defect created by the absence of a nucleotide on one strand compared with mismatched bases opposed to one another. Even single base-pair variance between heteroduplexes may be distinguished from homoduplexes formed between wild-type strands by their differential migration on polyacrylamide gels and HDA is thought to have a sensitivity similar to SSCP for small DNA amplicons under 300 base pairs [34,39]. As HDA identifies mutant alleles on a different principle than SSCP, the same PCR products can be prepared for both types of electrophoretic systems allowing both analyses to be simultaneously carried out in a complementary manner.

The protein truncation test (PTT) was first described by Roest and colleagues [40] in 1993 for mutation analysis of the Duchenne Muscular Dystrophy (*DMD*) gene. RNA isolated from blood lymphocytes is reverse transcribed and amplified by PCR (RT–PCR). Nested PCR is then performed with a modified primer containing a T7-promoter and a eukeryotic translation initiation sequence which allows for transcription/translation of the PCR products. Protein products are analysed by gel electrophoresis. Essentially, deleterious mutations are detected as truncated proteins that are distinguished from their full-sized wild-type counterparts on the eletrophoresis gel. The site of the mutation is pinpointed by the size of the truncated product. Although technically more complicated and requiring the use of a more difficult RNA approach, PTT has several advantages over SSCP and HDA analysis as it permits comprehensive screening of large amplified fragments of up to 2000 bases, an attractive feature in genes as large and complex as *BRCA1* and *BRCA2*. Furthermore, mutations identified by PTT have immediate clinical relevance because a truncated protein has been produced. Nevertheless, PTT also has its limitations. The most important is that because of the small size of the majority of exons in *BRCA1* this technique is only widely used for exon 11 analysis (which represents only approximately 60% of the coding sequence). In addition, missense mutations, with no premature termination of the protein product, will not be detected by PTT.

Although the use of these mutation-screening techniques can detect the large majority of *BRCA1* and *BRCA2* mutations, some will be missed. This is especially so if only genomic DNA is available, which is the case for most studies. In particular, mutations affecting splicing, expression or stability of the RNA transcript may not be detected by standard mutational analysis as the coding portions of the gene may be normal. Those mutations that occur in the regulatory portions of the gene may require analysis of cDNA for their detection and patients not screened for these types of mutations may receive false-negative results [14]. In a mutation analysis of 37 families with 4 or more cases of breast or breast and ovarian cancer by Friedman and colleagues [30], no mutations were detected in the coding sequence of the *BRCA1* gene. Five families had mutations that affected either splicing or the stability of the *BRCA1* transcript. These mutations that were detected only by analysis of the cDNA, represented 17% of all mutations detected in this population, suggesting that a significant proportion of *BRCA1* mutations may be missed by screening methods limited to the coding sequence of the gene.

A further shortcoming of these mutation screening techniques is that they require DNA that is usually extracted from leucocytes obtained from a blood sample. A living affected relative is therefore needed before these methods can be used to determine the *BRCA1* or *BRCA2* carrier status of a family. Mutation detection from archival tissue, such as stored paraffin blocks, is not widely available due to the fragmentation of the genetic material that occurs in the fixation process.

8. Mutation spectrum

BRCA1 is a large gene consisting of 5592 nucleotides spread over 100 000 bases of genomic DNA composed of 24 exons that encode a protein containing 1863 amino acids [14]. Much of *BRCA1* shows no homology to other known genes with the exception of a 126 nucleotide sequence at the amino terminus that encodes a ring finger motif. This is found in other proteins that interact with nucleic acids and form protein complexes, and suggests a role for BRCA1 in protein transcription. Shattuck-Eidens and associates [41] conducted a survey of the *BRCA1* mutation spectrum based on 63 mutation carriers identified using SSCP assays of the entire coding region of the gene. Thirty-eight distinct mutations were found, of which 86% were frameshift, nonsense or regulatory mutations that resulted in a truncated protein product. Analysis of the mutation spectrum revealed no evidence of clustering with an even distribution of mutations throughout the gene. This contributes to the difficulty in mutation screening, as analysis of the complete coding sequence is required for a thorough screen. Mutational analysis of the *BRCA1* gene is therefore laborious and time consuming. This contrasts with other genetic susceptibility genes such as the adenomatous polyposis coli (*APC*) gene. Although over 300 *APC* mutations have been found, they are almost all in the 5′ half of the gene, with two hotspots (codon 1061 and 1309) accounting for 15–20% of all cases [42,43], allowing rapid screening.

As previously stated, the size of the *BRCA1* gene has limited the use of direct sequencing as a method of mutational analysis in outbred populations [44–46]. However, Shattuck-Eidens and associates [47] reported the results of an international collaborative study in which the complete sequence analysis of the *BRCA1*

coding sequence and flanking intronic regions was carried out on 798 women. The study population consisted of affected representatives of families that were identified by high-risk clinics for features known to be associated with *BRCA1* germ line mutations. Of the 798 women analysed, 102 (12.8%) were found to have 48 different deleterious mutations, which were either truncating, known predisposing missense mutations or changes in conserved splice sites assumed to affect transcription splicing. Of the new deleterious mutations that had not been described by previous studies, only 33% occurred in exon 11, which represents 61% of the protein coding potential. This study demonstrated that mutation analyses which concentrate on exon 11 for its size and ease of amplification would miss a significant number of mutations affecting the remaining 23 smaller exons that require considerably more effort to screen.

Different strategies of mutation screening all employ the same preliminary step of PCR amplification of the DNA template. One shortcoming of this approach is that if there is a major rearrangement of the gene, only the wild-type allele would be amplified and as such, these rearrangements would be undetected. The first report of such a rearrangement was by Puget and associates [48]. The family contained 14 cases of breast cancer and 11 cases of ovarian cancer and had a lod score of 3.62 using two markers that flank *BRCA1*, yet SSCP analysis and sequencing of each exon of *BRCA1* had shown no evidence of any mutation. However, cDNA synthesised from RNA extracted from an immortalised ovarian cell line from an affected member of the family revealed a transcript, at a reduced level, without exon 17. Further analysis confirmed the deletion of a 1008 bp fragment encompassing exon 17. The significance of such germ line rearrangements was underlined by Petrij-Bosch and associates [49]. Four families which showed strong evidence of linkage to *BRCA1,* but no deleterious mutations following PTT of exon 11 and direct sequencing of all other coding exons and immediate intronic sequences were selected for RT–PCR. This analysis identified additional bands in two of these families. Sequencing of these products revealed a deletion of exon 22. Subsequent RT–PCR and Southern blotting of affected cases from 170 high-risk families detected the same exon 22 deletion in 15 cases and 7 cases had two large deletions encompassing exon 13. These large genomic deletions therefore accounted for 36% of all *BRCA1* deletions in this Dutch population study, suggesting the possibility of a founder effect. All were previously undetected using standard mutation detection methods.

Like *BRCA1*, *BRCA2* is a large gene, consisting of 27 exons spread over approximately 70 kb of genomic DNA that encodes a transcript of 12 kb to produce a protein of 3418 amino acids. *BRCA2* similarly shows no homology to other known proteins, although *BRCA2* exon 3 does show homology to c-Jun, a known transcription factor. Like *BRCA1*, most of the mutations detected in BRCA2 induce protein truncations that presumably lead to loss of the protein function [50]. They are also distributed evenly along the gene. Frank and colleagues [51] conducted a mutational analysis of 238 women with breast cancer diagnosed under 50 years or ovarian cancer at any age, all of whom also had at least one first- or second-degree relative for either diagnosis. Of the 31 women who were found to have deleterious *BRCA2* mutations, only 3 were found to occur more than once.

9. Founder effect

The proportion of high-risk families that are associated with *BRCA1* and *BRCA2* mutations varies widely among different populations. In most tested populations, *BRCA1* mutations have been found more commonly than *BRCA2*. *BRCA1* mutations are most commonly found in Russia (79% of breast–ovarian families) [52] but are uncommon in Japan (10%) [53]. There is also variation in the population dynamics of *BRCA1* and *BRCA2* in different countries, reflecting the historical influences of migration and cultural and geographical isolation. Most of the mutation-carrying families in Russia arise from two mutations (5382insC and 4135delA) [52] and a similar situation is found in Israel, where genotyping for ancient mutations found that only three BRCA1 mutations account for nearly all *BRCA1* Jewish families [54]. In contrast, nearly all mutations of *BRCA1* families in Italy are unique [55,56].

All germ line BRCA mutations identified to date have been inherited (with few exceptions only women with significant family histories have been studied), suggesting the possibility of a large 'founder' effect in which a certain mutation is common to a well-defined population group and can theoretically be traced back to a common ancestor. Given the complexity of mutation screening for *BRCA1* and *BRCA2*, these common mutations may simplify the methods required for mutation screening in certain populations. Analysis of mutations that occur with high frequency also permits the study of their clinical expression.

The most striking example of a founder mutation is found in Iceland, where a single *BRCA2* (999del5) mutation accounts for virtually all breast–ovarian cancer families [57,58]. This frameshift mutation leads to an early termination of codon 273 and a highly truncated protein product. To estimate the gene frequency of this mutation in the Iceland population, Thorlacius and associates [58] obtained DNA samples from 632 consecutive cases of invasive breast cancer, 520 unaffected control individuals unselected for gender or family history of cancer and 30 cases of male breast cancer. The

999del5 mutation was found in 0.6% of the general population, 7.7% of female breast cancer patients and 40% of male breast cancer patients. The same mutation was found in 24% of all female breast cancers under the age of 40 years. The high frequency of this mutation in different breast cancer families suggests a founder effect. This hypothesis was supported by the same pattern of DNA markers flanking the mutated *BRCA2* gene among apparently unrelated subjects. Interestingly, there was also a trend towards decreasing age of onset of cancer among carriers from successive generations of the same family. In addition, while 44 of the 61 patients who were found to be carriers had a moderate or strong family history of breast cancer, 17 had little or no family history of the disease. This is taken to be strong evidence for the presence of modifying genes that affect the phenotypic expression of this mutation, or possibly the interaction of the *BRCA2* mutation with environmental factors.

The most thoroughly studied manifestations of the founder effect are among Ashkenazi Jews. Four mutations in *BRCA1* and *BRCA2* have been reported to account for the majority of Ashkenazi Jewish patients with inherited breast and/or ovarian cancer: 185delAG, 188del11 and 5382insC in the *BRCA1* gene [30,44,59–62], and 6174delT in *BRCA2* [63]. The 185delAG mutation in exon 2 was the commonest mutation reported in a collaborative review of the mutation spectrum of the *BRCA1* gene by Shattuck-Eidens and colleagues [41]. Its association with individuals of Ashkenazi Jewish descent was first documented by Tonin and colleagues [61], who reported the mutation in 6/24 breast–ovarian cancer families, all of whom were Ashkenazi Jewish in origin. Berman and colleagues [62] studied 163 women from breast-ovarian cancer prone families and 178 individuals affected with breast and/or ovarian cancer unselected for family history. Fifteen 185delAG mutation carriers were found, of which 13 occurred in individuals of Ashkenazi Jewish descent. Haplotype analysis of these 13 families revealed the same pattern of DNA markers flanking the *BRCA1* gene, suggesting a common ancestor. As 2 of the 15 women could not be linked with this ancestor this provided the first evidence of at least two origins for the 185delAG mutation, only one of which arose in Ashkenazi Jews. The same mutational analysis also showed a second commonly occurring mutation (188del11), which was found in 10 affected individuals, of which 4 were Ashkenazi Jews and shared a common haplotype. Ethnic subgrouping was found to assist in identifying carriers of these mutations in families with unremarkable cancer histories. 6 out of 24 patients (25%) with breast and/or ovarian cancer and Ashkenazi ancestry were found to be carriers (two with 185delAG and four with 188del11). Only 1 of the 6 was later found to have a significant family history of cancer. Of the remaining 5 mutation carriers, 188del11 was found in 3 cases of late onset cancer, all of whom had breast cancer diagnosed in their 80s and had unremarkable family histories.

The 6174delT mutation in *BRCA2* was first detected in a breast–ovarian cancer family of Ashkenazi Jewish descent. In order to determine the frequency of this mutation, Neuhausen and colleagues [63] assembled a study population of 107 Ashkenazi women with breast cancer diagnosed before the age of 50 years, each of whom had a family history of a first- or second-degree relative with breast or ovarian cancer. Controls consisted of 93 cases of non-Jewish women. Eight 6174delT mutation carriers were found (7%), none in the controls. Combining this with previous data concerning 185delAG mutations in this same cohort of patients, the 185delAG and 6174delT mutations were together found to account for approximately two-thirds of all Ashkenazi Jewish individuals with early-onset breast cancer who had a personal or family history of ovarian cancer.

The identification of these 'common' mutations in Ashkenazi Jews allowed more population-based prevalence estimates of mutation frequency to be carried out. In an analysis of 858 Ashkenazi Jewish women seeking genetic testing for inherited conditions unrelated to cancer and unselected for family history of breast cancer, Struewing and associates [59] detected the *BRCA1* 185delAG mutation in 0.9%. This is 2 logarithms higher than the expected frequency of all *BRCA1* mutations combined in the general population. Controls consisted of 815 individuals not selected for ethnic origin and no *BRCA1* mutations were found. A similar study was carried out by Oddoux and colleagues on 1255 Ashkenazi Jewish individuals, again unselected for previous or family history of cancer and an identical prevalence of the *BRCA2* 6174delT mutation (0.9%) was found [64]. Roa and colleagues [65] conducted a population-based study consisting of 3000 individuals of Ashkenazi descent who had previously participated in other genetic studies and were unselected for cancer, as well as a mixed-ethnic control population of 1000 American individuals. The *BRCA1* 185delAG mutation was found in 1.09% and the *BRCA2* 6174delT mutation in 1.52% of the study population and in none of the control samples. Using these prevalence estimates and the age-specific penetrance risks compiled by the BCLC [20] (which are based on cancer incidence in large, high-risk families), the contribution of 185delAG to Ashkenazi Jewish women with breast cancer under the age of 50 years is approximately 20%. Although no age-dependent penetrance estimates were available for the *BRCA2* gene, indirect comparison suggested that the penetrance of 185delAG is approximately four times that of 6174delT, showing that different mutations may be associated with different risks of breast cancer.

Table 5 lists the founder mutations described to date although many others are likely to be identified.

Table 5
Founder mutations in *BRCA1* and *BRCA2*

Population subgroup [Ref.]	Mutation
Ashkenazi Jewish [31,44,59–63]	185delAG, 188del11, 5382insC.6174delT[a]
Austria [66]	2795delA, Cys61Gly, 5382insC, Q1806stop
Canada [67]	C4446T8765delAG[a]
Holland [49]	Exon 2, exon 13 deletion
Iceland [57,58]	999del5[a]
Norway [68,69]	1675delA, 1135insA
Poland [70]	5382insC, C61G, 4153delA
Russia [52]	5382insC, 4153delA
Scotland [71]	2800delAA

[a] *BRCA2* mutations.

10. Other cancers associated with *BRCA1* and *BRCA2*

BRCA1 and *BRCA2* carriers have been found to have an increased risk of other primary cancers. As already described, the most common is ovarian cancer. The penetrance of mutation in *BRCA1* and *BRCA2* genes for ovarian cancer has been studied in large families (Tables 2–4).

The predisposition of BRCA mutations to other cancers has been less well documented. In a study of 33 *BRCA1* families, Ford and associates [72] found the relative risk for colon and prostate cancers to be 4.11 (95% CI 2.36–7.15) and 3.33 (95% CI 1.78–6.20), respectively. No significant increased risk for other primary cancers was noted. Interestingly, all 17 colon cancers occurred in just 11 of the families, suggesting some heterogeneity in the colon cancer risk but supporting evidence for this is lacking. The study population consisted of large families with multiple relatives affected with breast and/or ovarian cancer, and studies on less distinctive pedigrees have not shown such high levels of either colorectal or prostate cancer risk. Struewing and associates studied the prevalence of colon cancers among relatives of 120 carriers of the *BRCA1* Ashkenazi Jews founder mutation [26]. Five per cent of carriers reported a case of colorectal cancer among their first- and second-degree relatives, compared with 11% of non-carriers. The only available study of colorectal cancer incidence among *BRCA1* carriers in an outbred population was recently reported by Lin and colleagues [73]. In a retrospective cohort study, the lifetime colorectal cancer risk in 163 known *BRCA1* mutation carriers was compared with that of the general population. No difference in the lifetime risk was found between the three groups (5.6% versus 6.0% for males and 3.2% versus 5.9% for females). Johannsson and colleagues [74] analysed the incidence of other cancers confirmed by local Cancer Registries among 1873 individuals of 29 *BRCA1* and 20 *BRCA2* families in Southern Sweden and found no increase in either colorectal or prostate cancers when the index cases were excluded.

To assess the association between *BRCA1* mutations and prostate cancer, Langston and colleagues [75] performed a case–controlled study of 49 men with prostate cancer which was likely to be genetic (age of onset of under 53 years, and a family history of a first degree relative with breast cancer diagnosed under the age of 51 years or two or more male relatives with prostatic cancer under the age of 56 years). Following mutation analysis with SSCP, only one deleterious mutation and four rare sequence variants were detected in the study population, suggesting that *BRCA1* has a minor role to play even in a selected subpopulation of prostate cancer patients. The only mutation detected in this study was 185delAG in a man of Jewish descent. Lehrer and colleagues [76] did not find any 185delAG *BRCA1* founder mutations in 80 Ashkenazi Jewish men with prostate cancer. *BRCA1* mutations therefore appear to make little contribution to cancer risk aside form breast and ovarian cancer.

The situation contrasts remarkably in the case of *BRCA2* mutations, which have been linked to cancers of the pancreas and prostate [77], as well as ocular melanoma [33]. The correlation to pancreatic cancer is particularly intriguing as biallelic somatic loss of *BRCA2* had been found in these tumours [16]. The first report of cancers other than breast and ovarian in *BRCA2* families was by Easton and colleagues [33] in two large *BRCA2*-related families that were systematically followed-up over four decades. Excesses of prostate and laryngeal cancer, while formally significant, were based on small numbers (two and five possible carriers, respectively). In a similar study of 49 extended families with site specific hereditary breast cancer, Phelan and associates [78] found a significantly higher incidence of pancreatic cancer in *BRCA2*-related families (4/8) compared with those families for which no mutations were found (5/41). The pancreatic cancers also occurred at a significantly earlier age than expected, further suggesting a genetic contribution. No significantly increased rates of other primary cancers were found in either of these studies. The issue of other cancer risk in *BRCA2*-related families was recently reviewed by the BCLC [79]. Three hundred and thirty-three cancers were found in 173 breast–ovarian cancer families identified at 20 centres in North America and Europe. An increased risk of pancreatic cancer was found (RR = 3.51, 95% CI 1.87–6.58) with carriers estimated to have a 2.1% cumulative risk of pancreatic cancer by the age of 70 years. The late onset of and relatively low penetrance of pancreatic cancer has been postulated by Goggins and assicuates [80] as being due to late inactivation of *BRCA2* in pancreatic cancer development. In an earlier study [81], 7% (5 cases) of apparently sporadic pancreatic cancer had been found to harbour *BRCA2* germ line mutations. The tumours of these patients had lost the wild-type *BRCA2* allele. There have been suggestions that *BRCA2*

mutation screening may be indicated for patients with pancreatic cancer, especially in the presence of a family history of breast and pancreatic cancers [82]. In a study of 102 histologically proven pancreatic cancers unselected for age or family history, Lal and colleagues [83] found three *BRCA2* mutation carriers, although all mutations were identical (6174delT) and all carriers were Ashkenazi Jews. Two of the three carriers had a family history suggestive of HBOC, and the third was adopted.

The association between *BRCA2* mutation carriers and prostate cancer is more debatable. Based primarily on family history from the index case, an increased risk of prostate cancer was found in putative carriers in the BCLC study [79] (RR = 4.65; 95% CI 3.48–6.22). Using cancer registry data in Iceland, Thorlacius and colleagues [57] found 12 close relatives of 61 carriers of the founder 999del5 mutation with prostate cancer (RR = 3.46; 95% CI 1.83–5.81). However, no evidence of founder *BRCA2* mutations were found in two separate series of familial and early onset prostate cancers in Ashkenazi Jews [84,85]. To maximise the likelihood of detecting BRCA mutations, Sinclair and colleagues [86] screened familial prostate cancer patients with families containing at least 3 cases of prostate cancer, 2 cases of breast and/or ovarian cancer for mutations at both BRCA genes. No truncating BRCA mutations were found, suggesting that BRCA mutations have a minor role to play in families with both familial prostate and breast cancers.

11. Phenotypic heterogeneity

Epidemiological evidence has suggested that families linked to the *BRCA1* gene may be divided into two variants based on their relative risks for ovarian cancer. Easton and colleagues [25] studied the incidence of breast and ovarian cancer in 33 families with linkage to *BRCA1*. A significant heterogeneity of cancer risk was found and the best fit to the data was obtained by assuming that there were two *BRCA1* alleles with different penetrance for breast and ovarian cancers. Families with a higher penetrance of ovarian cancer had a cumulative risk of 84% by 70 years of age, compared with 32% for lower penetrance families.

Early analysis of germ line *BRCA1* mutations suggested a correlation between mutations that occur at the 5′ end of the gene and increased risk of ovarian cancer. After characterising nine mutations detected by SSCP from 63 breast and 10 ovarian cancer patients from 10 *BRCA1* families, Friedman and colleagues [87] reported that four families with both breast and ovarian cancers had chain-terminating mutations occurring in the 5′ half of the *BRCA1* gene. In 10 *BRCA1* mutations detected in Finland, Vehmanen and colleagues [88] found that in five families with mutations in exon 11, nine breast cancers and 10 ovarian cancers were found, while in families with mutations downstream of exon 11 there were 19 breast cancers but only two ovarian cancers. Shattuck-Eidens and associates [41] classified families as having a high proportion of ovarian cancers if there was a minimum of 3 cases of ovarian cancer and the breast:ovarian cancer ratio was no more than 2:1. Families with at least 3 cases of breast cancer and in which the breast:ovarian cancer ratio was more than 2:1 were considered to have a low proportion of ovarian cancer. Only 4 of the 16 (25%) high ovarian proportion families had mutations at the 3′ third of the gene, while 16/31 (52%) of the low ovarian families had mutations in the same region ($P = 0.08$). Similarly, Gayther and colleagues [89] found that for 22 different mutations detected in 32 families that contained 86 cases of confirmed breast cancer under the age of 60 years and 76 cases of ovarian cancer, the ratio of breast to ovarian cancers and the site of the mutation was statistically significant ($P = 0.01$). The presence of a 'change point' was suggested, estimated to lie in exon 13 on each side of which the phenotype of mutations tended towards breast or ovarian cancer.

The presence of such genotype–phenotype correlations for *BRCA1* mutation carriers could have a profound influence on genetic counselling, cancer screening and prophylactic surgery. Unfortunately, other reports have failed to confirm the finding. Of 16 *BRCA1* families reported by Serova and colleagues [90], there was no association between mutation site and risk of ovarian cancer. Indeed, a single family that contained 9 cases of breast and 10 cases of ovarian cancer carried a mutation that leads to a truncated protein of almost full length. Frank and colleagues [51] analysed the mutation spectrum of 63 *BRCA1*-related cancers and showed no correlation between ovarian cancer and mutation site. The Ashkenazi founder mutations 185delAG and 10 188del11 are both in exon 2 and at the 5′ end of the gene and result in a similar truncated protein product. Yet, when the pedigrees of 25 carriers were analysed, 185delAG families were found to have a high proportion of ovarian cancers (42%), while 188del11 families had a low ovarian cancer prevalence (< 5%). There is therefore no consistent evidence for a genotype–phenotype correlation for *BRCA1* mutation carriers.

A genotype–phenotype correlation is also debated for *BRCA2*. Gayther and colleagues [91] found that among 22 deleterious *BRCA2* mutations identified in 25 families, there was an even distribution of breast alone and breast and ovarian families in the study cohort, but the mutations that occurred in families with a high proportion of ovarian cancers appeared to cluster in a region of approximately 3.3 kb in length in exon 11. Families with mutations in this region had 23 ovarian cancers and 18 breast cancers compared with 1 ovarian cancer

and 91 breast cancers in families with mutations that occurred elsewhere (odds ratio 116). In an international collaborative study [92] to determine the significance of this Ovarian Cancer Cluster Region (OCCR), Neuhausen and colleagues studied the ratio of breast to ovarian cancers in breast/ovarian cancer families having one of nine different *BRCA2* mutations. Four of these mutations were within the OCCR and five were outside the OCCR. Of the 82 families with mutations within the OCCR, the breast:ovarian cancer ratio was 160:48, while the 28 families with mutations outside the OCCR had a ratio of 103:14. This was not found to be statistically significant ($P = 0.12$) and more studies are required to establish whether a clinically useful clustering of ovarian cancer predisposing mutations does exist.

12. Pathobiological correlation

Loss of heterozygosity at the *BRCA1* and *BRCA2* loci in familial breast cancers suggests that these genes function as tumour suppressors. In carriers of inherited, or germ line mutations, cancer predisposition arises as a consequence of an acquired, or somatic mutation in the remaining (normal) copy of the gene. Surprisingly, somatic mutations in *BRCA1* and *BRCA2* are rare in sporadic breast cancers [93,94]. This difference between the pathogenesis of sporadic and hereditary breast cancers suggests that there may also be variations in their phenotype and clinical behaviour. Indeed, there is emerging evidence, which suggests that *BRCA1* and *BRCA2*-related breast cancers have specific morphological and prognostic features. Several studies have reported an association between *BRCA1*-related cancers and high tumour grade [95–98]. Two studies suggest that this is largely due to a correlation with high mitotic rate [96,97]. An association with typical medullary cancers has also been suggested [99]. A review by the BCLC studied breast cancers from 118 *BRCA1* and 78 *BRCA2* carriers among high-risk families. These were compared with 547 age-matched, randomly selected samples. Five pathologists who were blinded to the mutation status of each cancer carried out histological review of all slides independently. *BRCA1*-related cases had a clear association with higher mitotic counts and typical medullary or atypical medullary cancers ($P < 0.0001$) [100]. A second more recent histological review [101] asked two pathologists to specifically review features associated with medullary carcinomas. Three features were found to associate strongly with *BRCA1* tumours: high mitotic count ($P = 0.001$), continuous pushing margin ($P < 0.0001$) and lymphocyte infiltration ($P = 0.002$). Of these three features, the latter two are features of medullary carcinomas, although the other diagnostic features, such as vesicular nuclei, syncytial appearance and prominent nucleoli, were not independently associated with *BRCA1* mutations in this study. Furthermore, when typical and atypical medullary cancers were excluded from the analysis high mitotic counts, pushing margins and lymphoid infiltrate still remained significantly associated with *BRCA1*-tumours, suggesting that medullary carcinomas account for a small proportion of the differences between *BRCA1*-related and sporadic cancers. Mutation analysis of 42 cases of medullary and atypical medullary breast cancer at the Nottingham City Hospital Breast Unit detected 3 *BRCA1* mutations, but all carriers had either early-onset disease or a significant family history [102].

It has been reported that *BRCA1*-related tumours are less likely to have an *in situ* component than controls [96]. In a histological review of the distribution of ductal carcinoma *in situ* (DCIS) within and outside the tumour of 37 *BRCA1*-related cancers from 34 patients, Jacquemier and colleagues [103] found DCIS in 27% of hereditary cancers versus 56% of the controls (200 consecutive sporadic tumours). Tumours in *BRCA1* mutation carriers may rapidly obliterate their intraductal component because of their high proliferation rate. In a review of the incidence of DCIS in the Creighton University database of 36 *BRCA1*-related families, Sun and colleagues [104] found 202 cases of invasive cancer and only 4 cases of carcinoma *in situ* in these families. Of these four cases of *in situ* carcinoma, only 2 were mutation carriers. This suggests that *in situ* breast cancer may not be clinical evidence of the breast–ovarian cancer syndrome.

BRCA2-related tumours have also been associated with specific tumour types. In a double-blind study of 17 invasive cancers from 13 individuals, an excess of cancers in the 'tubular–lobular group' (TLG) was found [96,105]. This group, which has been associated with a more favourable prognosis, consists of invasive lobular, tubular, tubular–lobular and cribriform special type carcinomas. There was also an excess of lobular carcinoma *in situ* and atypical lobular hyperplasia. Similar histological features were found in nine *BRCA2*-related tumours detected in a population-based study of early-onset breast cancers reported by Armes and colleagues [99]. While these TLG carcinomas were proposed as signatures of *BRCA2*-hereditary breast cancer, the numbers reported were small and larger studies have failed to confirm the trend. The *BRCA2* phenotype may be more heterogeneous than that of *BRCA1*. Indeed, while recent investigations have suggested definite differences between *BRCA1*- and *BRCA2*-related breast cancers, *BRCA2*-related tumours may be more difficult to distinguish from sporadic cancers. In the BCLC pathological review [100], higher histological grade was recognised as a feature of *BRCA1* and *BRCA2*-related breast cancers, but *BRCA2* tumours had higher grade only because of decreased tubule formation ($P = 0.003$), showing no difference in pleomorphism or mitotic

counts. The differences in grading characteristics between *BRCA1*-related and *BRCA2*-related tumours were significant for pleomorphism ($P = 0.008$), mitotic count ($P < 0.0001$) and overall grade ($P < 0.0001$). There was also no evidence of any association between medullary cancer and *BRCA2* mutation status, and in contrast to earlier studies, none of the *BRCA2* carriers had tubular carcinomas. There is a possibility that the histological features of *BRCA2* tumours in this study may not be representative of all *BRCA2* tumours as 63% of the 78 cases studied were attributable to only two mutations in the *BRCA2* gene. In a study group consisting of 40 cases of breast cancer related to the Icelandic 999del5 *BRCA2* mutation, Agnarsson and colleagues [106] compared the histological features with 160 age-matched controls from the general population. 999del5-related tumours were found to have significantly higher grade due to less tubule formation, more nuclear polymorphism and increased mitotic frequency. There was no difference in histological type.

The steroid receptor status of *BRCA1* and *BRCA2*-related tumours is of particular interest in the light of the potential for preventive strategies for carriers. In the National Surgical Adjuvant Breast and Bowel Project P-1 Study [107], tamoxifen was found to decrease the incidence (or, more likely, delay the appearance) of oestrogen receptor (ER)-positive tumours by 69% after a median follow-up of 55 months. However, no difference in the incidence of ER-negative tumours was detected compared with controls. *BRCA1*-related cancers have been found in several series to have a low incidence of ER positivity (Table 6). While early-onset disease, high grade and poor differentiation are known to correlate with ER negativity, in a multivariate analysis of the morphological parameters of 32 *BRCA1*-related breast cancers and 200 consecutive tumours by Eisinger and colleagues [111], ER negativity was found to correlate independently of other histological factors. In contrast to *BRCA1*-related cancers, *BRCA2*-related cancers may be associated with ER positivity, suggesting that chemoprevention with ER modulators might have potential for these tumours (Table 6).

13. Survival

The survival of breast cancer patients with a family history of affected relatives has been the subject of several large studies. Most of these have been retrospective and variations in the definition of familiality, the choice of controls, method of statistical analysis and duration of follow-up have made comparisons difficult. Several studies have reported a survival analysis for known *BRCA1*-related breast cancer patients. Porter and colleagues reported better 5-year survival among 35 *BRCA1*-linked Scottish breast cancer patients compared with 910 age-matched controls (83% versus 61.1%) [114,115]. Unfortunately, staging information between the two groups was not provided and possible lead-time bias in the study group could not be excluded. Marcus and colleagues [96] analysed survival for 72 cases of histologically confirmed breast cancer who showed linkage to the *BRCA1* locus. Although there was a non-significant trend towards better crude survival in *BRCA1* mutation carriers, this was found to be age- and stage-dependent. Two case–controlled survival analyses, to date of *BRCA1*-related breast cancer patients confirmed on direct sequencing, are available. In the study by Verhoog and colleagues [109], each patient was matched with 4 cases of sporadic cancer for age, disease stage and date of diagnosis. No significant difference in menopausal status, operative procedure and stage of

Table 6
Studies reporting an association between BRCA mutations and oestrogen receptor (ER) status

Author	Study population	Control population	*BRCA1* carriers/controls ER +ve (%)	*P* value	*BRCA2* carriers/controls ER +ve (%)	*P* value
Johannsson [108]	*BRCA1* tumours from hereditary breast cancer families	*BRCA1*-negative tumours from hereditary breast cancer families	3 (8)/26 (68)	–	–	–
Karp [98]	Tumours from Ashkenazi *BRCA1* founder mutation carriers	Unselected *BRCA1*-negative tumours from Ashkenazi women	3 (23)/94 (74)	<0.001		
Verhoog [109]	Tumours from *BRCA1* hereditary breast cancer families	Age-matched sporadic breast cancers	9 (36)/98 (65)	0.002		
Osin [110]	Non-invasive portions (DCIS) of *BRCA1*- and *BRCA2*-related cancers	–	5 (33)/–			
Eisinger [111]	*BRCA1*-related tumours from hereditary breast cancer families	Sporadic cases without family history of breast cancer	3 (10)/130 (65)	<0.001		
Verhoog [112]	*BRCA2*-related tumours from hereditary breast cancer families	Age-matched sporadic cases	–	–	14 (93)/54 (84)	NS
Noguchi [113]	*BRCA1*- and *BRCA2*-related tumours from hereditary breast cancer families	Age-matched sporadic cases	3 (17)/48 (64)	<0.01	6 (60)/48 (64)	NS

NS, non-significant.

disease was found between the two groups. No difference in recurrence or survival was found, with the hazard ratio for recurrence and death among the *BRCA1* patients being 1.00 (95% CI 0.65–1.55) and 1.04 (0.63–1.71) relative to the sporadic cases ($P=0.88$). The relationship between *BRCA1*-related tumours and bilateral disease was confirmed by this study, with 25% of these patients developing contralateral breast cancer within 5 years of the initial diagnosis. In a similar study by Johannsson and colleagues [116], survival of 33 cases of known *BRCA1* carriers with breast cancer was compared with controls that were matched for age, stage, time of diagnosis and treatment. Again, survival of the mutation carriers appeared to be similar to controls (hazards ratio 1.5, 95% CI 0.6–3.7).

It therefore appears that *BRCA1*-related tumours are associated with high grade, but not necessarily poor survival. Some suggestions for this discrepancy have been put forward by Watson and colleagues [117]. *BRCA1*-related tumours might not represent typical high-grade breast cancer. The genetic instability in *BRCA1*-related tumours indicated by prevalent aneuploidy and increased p53 expression may suggest an increased susceptibility of these tumours to chemotherapy and radiotherapy. The incidence of c-*erb*B-2 overexpression, a marker that usually indicates poor prognosis found in the majority of high-grade breast cancer [118], is also no greater in *BRCA1*-related tumours than sporadic breast cancers [108]. The association between *BRCA1*-related tumours and medullary carcinomas, which are higher grade but have a favourable prognosis, may also partly explain this irregularity.

These case–controlled studies are all subject to selection bias towards greater survival in the *BRCA1*-related group [119,120]. This is because at least one affected relative in the multiple cancer groups has to be alive in order for genetic testing to be conducted, whereas control patients from cancer registries need not be alive to be included in the study. Furthermore, as controls were not screened for *BRCA1* mutations, the age-matched controls can be expected to include some *BRCA1* mutation carriers as well, although the effect of this on the analysis is unknown. In this regard it is interesting to note that there is a trend towards poorer survival in the BRCA1 group in the Verhoog study [109] when the probands are excluded. Foulkes and colleagues [121,122] overcome this bias by using a historical cohort approach, where *BRCA1* mutation status was determined among unselected cases of breast cancer in Ashkenazi women by mutation analysis of DNA derived from tumour blocks. A total of 118 tumours of women with node-negative cancers were examined for the Ashkenazi founder mutations and 16 carriers were found. Following multivariate analysis of conventional prognostic factors, only germ line *BRCA1* mutation status was found to be an independent prognostic factor of poor survival ($P=0.01$). The only survival analysis of *BRCA1* mutation carriers unselected for family history in an outbred population was reported by Ansquer and colleagues [123]. Mutation analysis of the *BRCA1* gene in 123 women treated at the Institute Curie in Paris for breast cancer diagnosed under the age of 36 years detected 15 deleterious mutations. Compared with women in whom no deleterious mutations were found, *BRCA1* carriers were noted to have tumours of higher grade, ER and progesterone receptor (PR)-negativity, and a greater incidence of metachronous and synchronous contralateral tumours, in keeping with earlier studies. However, at a mean follow-up of 43 months (range: 4–93 months), the overall survival in BRCA1 mutation carriers was noted to be worse compared with women in whom no mutations were found ($P<0.04$). In a similar study by Robson and colleagues [124], 91 women of Ashkenazi Jewish descent with breast cancer diagnosed under the age of 42 years and unselected for family history underwent mutation analysis for founder mutations in *BRCA1* (185delAG and 5382insC) and 79 were also tested for the founder *BRCA2* mutation, 6174delT. Mutations at these sites were noted in 30 (33%) of the women tested. No significant difference was noted between the 5-year overall or relapse-free survival of the mutation carriers compared with non-carriers, although both *BRCA1* and *BRCA2* mutation carriers were analysed together.

Due to its later discovery, less is known about the prognosis of cancers that arise in *BRCA2* carriers. However, recent publications have shown a similar survival between *BRCA2*-related and sporadic cancers. Verhoog and colleagues [112] analysed the survival status of 28 cases of breast cancer drawn from 14 *BRCA2* families. Each case was matched for age and year of diagnosis (but not stage) with 4 cases of sporadic cancers from the hospital's cancer registry. At 5 years, there was no difference in overall survival or disease-free survival. While *BRCA2*-related tumours were not significantly larger, axillary nodal status was no different between the two groups. As with *BRCA1*-related tumours, contralateral breast cancers were significantly more common in the *BRCA2* group (12%) compared with 2% in controls ($P=0.02$).

14. Ataxia-telangiectasia

Ataxia-telangiectasia (AT) is a rare, fully penetrant autosomal recessive syndrome present in 1/40 000 to 1/100 000 live births characterised by progressive cerebellar ataxia and oculo-cutaneous telangiectasia. Ataxia is progressive from infancy, while telangiectasias may take years to develop. Homozygotes for the mutated *AT* gene (*ATM*) have a 100-fold increased risk for developing cancer and are markedly radiosensitive, with thera-

peutic irradiation often producing devastating necrosis of normal tissue. Only limited data are available for estimating the frequency of *ATM* as newborn or population screening is not yet possible. However, based on two case-finding periods in 1970–2 and 1980–4 among all registered paediatric neurologists in the USA, Swift and colleagues [125] accumulated a study population of 263 homozygotes from 189 families. The majority were Caucasian and of European descent. From the number of cases identified by this study, the minimum incidence of *AT* homozygotes was estimated at 3.0 per million live births. Using pedigree analysis, which estimates carrier frequency from the proportion of affected close blood relatives of the homozygous proband, the estimated frequency of *ATM* was 0.007 (95% CI 0.002–0.02). Based on this figure, the heterozygote frequency was calculated to be 2.8% of the general population (95% CI 0.68–7.7%). This was thought to be the lower limit of the true frequency as not all neurologists had responded to the survey and a significant number of *AT* homozygotes are not diagnosed until late in the first or even the second decade of life. Variations in clinical manifestations also mean that an unknown proportion die without diagnosis.

Based on 110 families obtained in the second case-finding period (1980–4), Swift and colleagues [126] carried out a retrospective analysis of cancer rates in blood relatives and obligate *AT* heterozygotes compared with spousal controls. The relative risk of developing cancer for heterozygous adults was elevated for both males (2.3, $P=0.014$) and females (3.1, $P=0.004$). The most frequent association was with breast cancer, for which the relative risk compared with controls was 6.8 ($P=0.006$). Interestingly, the relative risk was greatest in the 45–54 year age group rather than for very young women (7 versus 1.6 cases per 1000 person-years). This predisposition to breast cancer formation in *ATM* heterozygotes has been supported by other studies, although the confidence limits of the relative risk estimates are wide [127,128]. An independent review of early studies was carried out by Easton [129] and suggested that *AT* heterozygotes account for between 1 and 13% of all breast cancer, with the best estimate being 3.8%. The risk of breast cancer development in *AT* heterozygotes has been estimated at 11% by age 50 years and 30% by age 70 years [130]. The relative risk of developing cancer for heterozygous adults was elevated for both males (2.3, $P=0.014$, goodness-of-fit $P=7.74$) and females (3.1, $P=0.004$, goodness-of-fit $P=0.74$). The most frequent association was with breast cancer, for which the relative risk compared with controls was 6.8 ($P=0.006$ goodness-of-fit $P=0.24$).

Genetic haplotyping of these *AT* families localised the *AT* gene to chromosome 11q23 [131] and it was subsequently cloned by Savitsky and colleagues in 1995 [132], allowing genetic analysis to identify putative *AT* heterozygotes. In support of earlier studies, genetically confirmed *AT* heterozygotes were also found to have a higher incidence of breast cancer development. Athma and colleagues [133] carried out molecular genotyping of breast cancer cases in relatives of *AT* homozygotes. Using markers which closely flank the AT gene locus, 775 blood relatives from 99 *AT* families were genotyped. Among these relatives were 33 women with breast cancer. Of these women, 25 were found to be heterozygotes, significantly in excess of the expected 14.9 (odds ratio 3.8, $P=0.0001$). When compared with genotyped non-carriers from the same families, the odds ratio for 21 cases of breast cancer before age 60 years was 2.9 (1.1–7.6, $P=0.009$) and for 12 cases aged 60 years and over, the odds ratio was 6.4 (1.4–28.8, $P=0.002$). These results suggest that *AT* heterozygosity tends to predispose to relatively late-onset breast cancer. The authors also calculated that *AT* heterozygotes account for 6.6% of all breast cancers in the USA. Later studies of *AT* families in the UK [134,135] and France [136] have supported these findings although the confidence intervals are wide due to the small size of the study populations.

Although there appears to be consistent evidence of increased breast cancer risk in *AT* heterozygotes, identifying such individuals may be difficult as familial clustering of cases will be less prominent than for *BRCA1* and *BRCA2* mutation carriers due to the relatively low breast cancer penetrance. Nevertheless, given the relatively high prevalence of the ATM gene in the general population, one might expect a significant number of breast cancer cases to carry the *ATM* gene. Interestingly, evidence of this has not been readily forthcoming. In a germ line mutational analysis of the *AT* gene performed using the protein truncation test (PTT) on blood samples from 401 women diagnosed with breast cancer before the age of 40 years, Fitzgerald and colleagues [137] found only two *ATM* carriers. Vorechovsky and colleagues [138] carried out loss of heterozygosity (LOH) analysis at the *AT* locus in 38 consecutive cases of apparently sporadic breast cancer with a mean age of onset of 55.8 years, followed by mutational analysis of the *AT* gene. While 47% (17/36) of the informative cases were found to have LOH at one or more of the four markers flanking the *AT* gene, SSCP analysis failed to detect any deleterious mutations in the gene in any of the cases. The presence of LOH in the markers in this region suggests the presence of a tumour suppressor gene in the vicinity of, but possibly not at, the *AT* locus. Using the same mutation screening methods, Vorechovsky and colleagues [139] then analysed 88 unrelated index cases of breast cancer who each had a family history of cancers associated with *ATM* to determine if carriers have an increased cancer susceptibility. At least 1 additional case of breast cancer, lymphoma, leukaemia or gastric cancer needed to be present among close family members. Only 3 women were found to be *ATM*

carriers, each of whom had families with a large number of tumours. However, analysis of the affected relatives found that mutations did not cosegregate with the cancers, thus providing no evidence to support increased cancer susceptibility in carriers.

Recent developments might explain some of the seemingly contradictory findings on cancer risk associated with *ATM* carriers. Meyn [140] has suggested that there may be clinical heterogeneity among *ATM* carriers, where distinct mutations in the *AT* gene may give rise to variable clinical expression. While 80–90% of mutations occurring in AT families give rise to a truncated protein product, missense mutations that lead to amino acid substitutions or deletions/insertions may be more common among patients with breast cancer. Such mutations would be missed on PTT analysis [137] because of the production of a protein of normal length. Interestingly, two families in the UK *ATM* study [135] were found to carry such a missense mutation (7271TΠG). Carriers in these families were found to have an increased risk of breast cancer (RR 12.7, 95% CI 3.53–45.9, $P=0.0025$), with less severe *AT* phenotype in terms of cerebellar degeneration. There is also a possibility that breast cancer in *ATM* carriers may have a distinct clinical phenotype. Broeks and colleagues [141] found deleterious *AT* mutations in 7/82 (9%) Dutch patients with specific characteristics. All patients developed had breast cancer under the age of 45 years, of which 40% had bilateral disease, and had survived a minimum of 5 years.

Swift suggested that since carriers of *ATM* form a sub-population which may be sensitive to relatively low radiation exposure, there was a legitimate concern that diagnostic radiation may contribute to some of the increased incidence of breast cancer in this group. This hypothesis has yet to be proved but it is interesting that 11 women in the study by Fitzgerald and colleagues [137] had received therapeutic radiation to the chest prior to developing breast cancer and none was found to be *ATM* carriers. In addition, there is no evidence of radiosensitivity among *AT* heterozygotes with breast cancer given adjuvant radiotherapy [138].

Due to the unconfirmed allele frequency of *ATM* in the general population, the small number of breast cancers detected in carriers (and vice versa), and the possibility of a spectrum of mutations in the gene, the real association between ataxia-telangiectasia and breast cancer may not be determined until large-scale population-based studies with adequate selection of cases and controls are conducted [142].

15. Li-Fraumeni syndrome

The Li-Fraumeni syndrome was first described in 1969 when Li and Fraumeni reviewed the medical records and death certificates of 648 childhood rhabdomyosarcomas [143]. They identified four families, in which there was a striking history of breast and other carcinomas, suggesting the presence of a familial cancer syndrome with diverse primaries. This suggestion was further supported by prospective follow-up of the survivors in these families [144]. Between 1969 and 1981, 10 of the 31 surviving relatives developed 16 additional cancers (expected = 0.5, $P=0.001$) including 8 patients who had multiple primary cancers. Most were under 35 years of age at diagnosis. The distribution of cancer in these families suggested an autosomal dominant mode of inheritance and predicted that 50% of gene carriers would develop invasive cancer by the age of 30 years, and 90% by the age of 70 years. The association between childhood soft tissue sarcomas and other cancers was confirmed in a segregation analysis by Strong and associates [145], who analysed the incidence of cancer in relatives of 159 patients with soft tissue sarcomas. Significant excess cancers were found in the 758 first-degree relatives and these were predominantly soft tissue sarcomas, bone and breast cancers.

The high mortality among carriers, lack of a distinct tumour type or pre-cancerous lesion and the rarity of the gene-hampered traditional linkage studies to identify the location of the genetic alteration in these families. Using the alternative strategy of screening for potential candidate genes, Malkin and associates [146] targeted *TP53*, as inactivating mutations in this gene had been identified with cancer types associated with the Li-Fraumeni syndrome. In 1990, evidence of germ line *TP53* mutations was found when DNA taken from fibroblasts of an affected member of a Li-Fraumeni family showed alterations in a normally highly conserved portion of the *TP53* gene. This was confirmed to be a germ line mutation when the same alteration was found in lymphocytes from the same subject, and when the same mutation segregated in fibroblasts of affected members of Li-Fraumeni families [146].

To assess the role of germ line *TP53* mutations outside the setting of high risk families, Sidransky and colleagues [147] conducted mutational analysis for 126 consecutive patients who had breast cancer before the age of 40 years. Forty-eight per cent of these women had a family history of cancer in one or more first-degree relatives, of whom a quarter had breast cancer. Only 1 of the patients studied was found to have an abnormal *TP53* allele. She had breast cancer diagnosed at the age of 33 years and subsequently developed melanotic spindle cell cancer of the mediastinum at age 35 years. Her family history consisted of postmenopausal unilateral breast cancer in her maternal grandmother and bilateral breast cancer in her mother. The detected *TP53* mutation was confirmed as germ line when her sister, who had undergone prophylactic bilateral mastectomies, was found to carry an identical mutation. Only one of the 136 unselected breast cancer cases

examined by Cole and colleagues [148] was found to carry a germ line mutation at the *TP53* gene, suggesting that germ line *TP53* mutations play a very minor role in sporadic breast cancers.

Recently, Bell and colleagues [149] screened four families with classical Li-Fraumeni pedigrees, but in which in no germ line *TP53* mutations were found, for germ line mutations at the *kChk2* gene. This gene is a human homologue for yeast *Cds1* and *Rad53* genes which encode G2 checkpoint kinases. One family was found to have a germ line mutation (1100delC) which led to a premature stop codon and which segregated with affected relatives. The phenotypic similarity to *TP53* mutation carriers suggested a common or similar function between the two tumour suppressor genes. This hypothesis was recently confirmed by Hirao and colleagues [150], who demonstrated *in vitro* that *Chk2* gene expression was required for p53-dependent transcriptions in response to gamma (γ) irradiation.

16. Cowden's disease

Cowden's disease is a rare inherited syndrome with a strong predisposition for breast cancer. Named after the first affected patient described in 1963 [151], it has an autosomal dominant pattern of inheritance and is characterised by hamartomas of ectodermal, endodermal and mesodermal origin. Also called multiple hamartoma or multiple hamartoma and neoplasia syndrome, Cowden's disease is characterised by facial and oral mucosal papillomatosis, acral and palmoplantar keratosis and a family history of the syndrome [152,153]. While thyroid disease in the form of adenomas is the most common internal manifestation of the syndrome [154], breast cancer has been seen in 30% of affected women with a median age of onset of 41 years [155]. The Cowden's disease susceptibility gene has recently been identified as the tumour suppressor gene *PTEN*, also known as *MMAC1* and *TEP1*, which maps to 10q23.3 and encodes a phosphatase 403 amino acids in length [156,157]. Germ line mutations at *PTEN* have also been found to be responsible for Bannayan–Ruvalcaba–Riley (BRR) Syndrome, another rare autosomal dominant neoplastic disorder characterised by microcephaly, vascular malformations and benign neoplasia [158]. Somatic mutations at *PTEN* have been identified in breast, prostate and endometrial cancers, among others [159–162].

The association between the *PTEN* gene and primary cancers not included within Cowden's or BRR syndromes suggests that the *PTEN* gene may function as a tumour suppressor, raising the possibility that germ line mutations may be involved in other sporadic cancers not associated with these syndromes. To test this hypothesis, De Vivo and colleagues [163] carried out germ line mutation analysis at the *PTEN* gene on 103 subjects from the Nurses' Health Study, all of whom had more than one primary tumour at different anatomical sites during the interval of study. Controls consisted of age-matched individuals who had not been diagnosed with cancer. Five deleterious mutations were found in study subjects and none in controls ($P = 0.02$), suggesting that germ line *PTEN* mutations may be a predisposing factor for multiple cancers in the absence of features suggestive of Cowden's or BRR syndromes.

17. Bloom syndrome

Equally uncommon is Bloom Syndrome, an autosomal recessive disorder that is due to homozygosity at *BLM*, a locus on chromosome 15 that has yet to be completely characterised. The underlying pathology appears to be a widespread genetic instability leading to an increased frequency of somatic mutations. As of 1993, only 165 persons had been recorded in the Bloom Syndrome registry [164]. The typical phenotype consists

Table 7
Commonly deleted regions and examples of candidate genes that map to these regions

Commonly deleted region	Gene(s)
1p36.1-36.2	*MDGI, BRCD2*
2q21.3-23.3	*FAP*
3p14.1	*FHIT*
5q21.1-21.3	*APC*
6q15	*MYB*
6q22.1-23.1	*MYB, ROS1*
6q25.2-27	*hZAC, ESR-1*
7q31.1-31.31	*CAV-1*
8p22-21.3	*N33*
8q24	*MYC*
9p21	*CDKN2A, CDKN2B*
10q23.31-23.33	*SNCG, PTEN*
11p15	*ST5, TSG101, HRAS*
11q13.1	*EMS1, INT-2*
11q23.3	*ATM*
13q12.3	*BRCA2*
13q14.2-14.3	*RB1*
14q32.11-31	*OGR1*
16q11.2-22.1	*CDH1*
16q22.3-24.3	*CDH13*
17p13.3	*BCPR*
17p13.1	*TP53*
17q21.23	*BRCA1*
18q21.1-21.3	*DCC*
22q12.3	*RRP22, NF2*
22q13.1	*ST13*

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FAP, familial adenomatous polyposis; *APC*, adenomatous polyposis coli; *CDKN*, cyclen dependent kinase; *ATM*, ataxia telangiectasia mutant; RB1, retinoblastoma; *DCC*, deleted in colon cancer.

of proportional dwarfism as well as severe immune deficiency and a predisposition to early-onset cancer. Of the 165 persons on the registry, 86 malignancies had been detected in 60 individuals, of which the commonest were leukaemias, lymphomas and colonic carcinomas. Of the 86 malignancies, 41 were carcinomas, of which 7 were breast cancers, which had a mean age of diagnosis at 32 years (range: 18–46 years).

18. Other mutations

Currently under investigation are many other genes and potential sites for genes in which germ line mutations may have an association with breast cancer susceptibility. These have not been discussed in this review. Table 7 lists commonly deleted regions identified by loss of heterozygosity in tumour-derived DNA and examples of candidate genes that map to these regions. Many of these regions do not contain genes for which a link with breast tumorigenesis has been firmly established, although candidate genes can be derived for them, some of which are also shown in the Table. The list of candidate genes is not exhaustive.

19. Conclusions

Advice regarding a family history of breast cancer is one of the commonest reasons for referral to a specialist breast clinic. The fast developing field of breast cancer genetics has therefore received considerable publicity and advances have been eagerly translated into clinical practice with confident expectation of clinical benefit. However, as this review shows, while great advances have been made in the past decade, there are many gaps in our knowledge. Mutations in *BRCA1* and *BRCA2* are undoubtedly clinically significant. They are highly penetrant and are prevalent within certain population subgroups, particularly those in which a founder mutation has been identified. They also account for a small, but significant, proportion of young-onset breast cancer, although at most this is 10–15%. Sense has yet to be made of the considerable heterogeneity of phenotype that is observed between different mutations and between carriers of the same mutation. Detailed studies are required on large numbers of carriers to elucidate these important areas. Until more accurate information is available the genetic advice given to any individual is based on figures with relatively broad confidence intervals. Technical advances in mutation analysis will, in the near future, allow for more accurate and rapid mutation detection and facilitate the study of these genes in large samples of the population. Such advances may also establish the clinical importance of less penetrant, but more prevalent, mutations such as *ATM*.

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