Recent advances in understanding of genetic susceptibility to breast cancer

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The breast cancer susceptibility gene, BRCA1, was isolated in 1994. Recent investigations into the genetic epidemiology of BRCA1 have revealed an unexpectedly high prevalence of mutations amongst Ashkenazi Jews. Analyses of BRCA1 function have indicated that it may act as an inhibitor of cell proliferation and is necessary for normal development in the mouse. The presence of a motif in BRCA1 characteristic of a family of proteins known as gramins, has led to the suggestion that the protein is secreted into the extracellular space. The BRCA2 gene has recently been identified. BRCA2 encodes a large protein of 3418 amino acids without strong homology to any other protein in the database. However, BRCA2 also contains a putative granin motif and a different eight times repeated motif of unknown function. In addition to breast and ovarian cancer, germline BRCA2 mutations probably confer a small risk of a wide range of cancers. Somatic mutations of BRCA2 in sporadic breast and ovarian cancer are very rare. The gene for Cowden syndrome has recently been located and it will now be possible to assess whether it is responsible for the set of families not accounted for by BRCA1 and BRCA2.

INTRODUCTION

A review of familial predisposition to breast cancer was published last year in the equivalent issue of this journal (1). This article will therefore mainly discuss observations that have been reported over the last 12 months.

BRCA1

The epidemiology of breast cancer susceptibility due to BRCA1

Following the isolation of BRCA1 in 1994 (2) and the description of many disease causing mutations in the gene, studies of the prevalence and penetrance of BRCA1 mutations in populations unselected for family history of breast cancer are now being reported. For example, 13% women diagnosed with breast cancer under the age of 30 and 7% diagnosed under the age of 35 have been found to carry BRCA1 mutations, results which are close to previous estimates (3–5). An unexpected finding, however, has emerged from study of Ashkenazi (Eastern European) Jews. Subsequent to the observation that several breast cancer families of Ashkenazi origin carry the BRCA1 mutation 185delAG, the frequency of this mutation was examined in a series of Ashkenazim seeking genetic testing for conditions unrelated to cancer. Surprisingly, just under 1% harbour this particular BRCA1 mutation, a prevalence 3–10 fold higher than previous estimates (6). An unexpected finding, however, has emerged from study of Ashkenazi (Eastern European) Jews. Subsequent to the observation that several breast cancer families of Ashkenazi origin carry the BRCA1 mutation 185delAG, the frequency of this mutation was examined in a series of Ashkenazim seeking genetic testing for conditions unrelated to cancer. Surprisingly, just under 1% harbour this particular BRCA1 mutation, a prevalence 3–10 fold higher than previous estimates (6). (Indeed, this turns out to be not the only BRCA1 mutation amongst Ashkenazim, as a mutation observed in other families of European origin, 5382insC, is also found at lower frequency.) Approximately 20% of Ashkenazi breast cancer cases diagnosed under age 40 are attributable to the 185delAG mutation (4,7). The fact that most carriers identified through screening of population based sets have affected first and/or second degree relatives, suggests that the penetrance estimates previously obtained by study of large families are more generally applicable (for this mutation at least) (4,7). Moreover it is likely that much of the excess risk of breast cancer amongst Ashkenazim compared to other ethnic groups is attributable to high prevalence of susceptibility gene mutations rather than environmental or lifestyle factors (8). These findings obviously have implications for presymptomatic diagnosis of susceptibility to breast cancer amongst Ashkenazim. A substantial component of high penetrance susceptibility to breast cancer can easily be detected in this population using probes to a few mutations and the mutations are common enough to consider offering such a test to women affected by breast cancer at an early age (or their close relatives) with or without a family history of the disease. The arguments for and against embarking on this course have been aired (9).

Genetic and environmental or lifestyle factors that may modify the risk of cancer in BRCA1 mutation carriers are now being studied. Low parity, which is a risk factor for sporadic breast cancer, appears to have a similar effect in carriers of BRCA1 mutations (10). It has also been proposed that rare alleles of the H-ras VNTR may be associated with an elevated risk of ovarian cancer in BRCA1 mutation carriers (11).

Correlation between the position of a mutation in BRCA1 and risk of cancer

Before BRCA1 was isolated, it was suggested that different mutations might confer different risks of cancer. Specifically, it was postulated that the majority of mutations confer a high risk of breast cancer but only a moderately elevated risk of ovarian cancer whilst the minority confer an equally high risk of breast and ovarian cancer (12). A biological mechanism that may
account for the existence of these two variants has now been proposed. Truncating \textit{BRCA1} mutations in families with a high proportion of ovarian cancers tend to be located in the 5′ two thirds of the gene whilst families with predominantly or exclusively breast cancer tend to have mutations in the 3′ one third of the gene (13). The most parsimonious hypothesis to account for this observation is that there exists a domain in the vicinity of amino acid 1400 (but which could be anywhere between 600 and 1600) of the \textit{BRCA1} protein which confers some protection against ovarian cancer. When the domain is removed by a truncating germine mutation the risk of ovarian cancer is high. When the domain is intact the risk of ovarian cancer is lower, but still in excess of that in the general population. Although other studies lend support to the hypothesis that there is a difference in ovarian cancer risk between different \textit{BRCA1} mutations, some detect the correlation between position of mutation and ovarian cancer risk whilst others do not (14,15).

The biology of \textit{BRCA1}

The primary structure of \textit{BRCA1} has yielded few clues to its function. A C3H4 RING finger domain is present towards the N-terminus of the protein, but the function of this particular subclass of zinc finger domain has not been well characterised in other proteins. Mouse \textit{BRCA1}, overall, shows only 58% amino acid identity to the human, considerably less than that observed in most other cancer susceptibility genes (16). Nevertheless, the RING finger region is highly conserved, adding weight to the suggestion that it is important in the function of the protein. Murine \textit{BRCA1} is expressed widely during development and is present in differentiating epithelial cells of several adult organs (17,18). \textit{BRCA1} mRNA levels rise markedly in murine breast epithelial cells during puberty and pregnancy and expression in human breast cancer cells is regulated by sex steroids (17-19).

A \textit{BRCA1} mouse homozygous knockout has been generated. This animal dies in utero with multiple developmental abnormalities of the central nervous system (20). This is in contrast to the only reported human with a homozygous \textit{BRCA1} mutation, who developed normally but was diagnosed with breast cancer in her thirties (21). The mouse heterozygote is viable but no tumours were reported at 6 months.

Several groups have developed antibodies against the \textit{BRCA1} protein which have generated conflicting and confusing results. One group concluded that the 220 kDa \textit{BRCA1} protein is located predominantly in the nucleus of normal cells but that in breast cancer cells the protein is in the cytoplasm (22,23). In the light of the absence of somatic \textit{BRCA1} mutations in sporadic breast cancers, this led to the exciting hypothesis that \textit{BRCA1} is often excluded from its appropriate target within breast cancer cells and, as a consequence cannot function.

However, others contend that the 220 kDa \textit{BRCA1} protein they detect is located in the nucleus of both neoplastic and non neoplastic cells (24) or that the protein is 190 kDa in size and is predominantly associated with membranes (notably Golgi and secretory vesicles) of normal and neoplastic cells (25). The latter group noted the presence of a short motif within \textit{BRCA1} identical to a sequence present within a family of proteins known as granins. Granins are acidic proteins that bind calcium, aggregate in its presence and are found in the trans Golgi network and secretory granules of cells. They are believed to play a role in the processing of proteins whose secretion is regulated by signals from the extracellular environment (26). Granins may themselves be cleaved into bioactive peptides. Although the granin motif is in an unusual position within \textit{BRCA1} and there is no signal sequence to facilitate transfer across membranes it has been suggested that \textit{BRCA1} may function as a granin and may even act as a secreted molecule binding to an extracellular receptor. The granin motif is in approximately the predicted position of the hypothetical domain which may confer partial protection against the development of ovarian cancer (13). Whether they are identical is a matter for speculation at present. Overall, however, the published evidence in support of \textit{BRCA1} being a granin and being secreted is at present circumstantial and the various sets of conflicting data will require reconciliation before further conclusions can be drawn.

Introduction of a normal \textit{BRCA1} cDNA expression construct into breast and ovarian cancer (but not other cancer or non neoplastic) cell lines results in reduction of proliferation and tumour forming ability (14). Although this is precisely the effect predicted if \textit{BRCA1} is a tumour suppressor gene, the interpretation of this experiment is complicated. Somatic mutations of \textit{BRCA1} are unknown in sporadic breast cancers and rare in sporadic ovarian cancers (27-30), therefore the cell lines used in these experiments already contain a normal \textit{BRCA1} copy. Does the introduction of a \textit{BRCA1} expression construct into these tumour cells increase the level of \textit{BRCA1} transcript and/or protein back to what it was in the normal progenitor cell from which the cancer arose or does it take it far in excess of normal levels? If the former is true, these results may indicate that reduction in \textit{BRCA1} expression is an important step in the development of sporadic breast cancer. If the latter is the case, the results may be artefacts of an artificially engineered system. Part of the problem is that we do not know whether all, some, or a small minority of cells within breast and ovarian epithelia can be progenitors of cancers and therefore the levels of \textit{BRCA1} expression in these critical target cells is also unknown. Moreover, studies of levels of \textit{BRCA1} protein in sporadic breast or ovarian cancer have been confounded by uncertainty over reagents. \textit{BRCA1} expression constructs lacking the C-terminal 28 amino acids lose the ability to suppress the growth of breast cancer cell lines but retain the ability to suppress the growth of ovarian cancer cell lines (14). These results parallel intriguingly the observation that individuals heterozygous for mutations that result in near full length truncated \textit{BRCA1} protein are at high risk for the development of breast cancer but cause only a low risk of ovarian cancer (13,14).

\textit{BRCA2}

The identification of \textit{BRCA2}

Evidence for a second major breast cancer susceptibility gene initially emerged from the analysis of 214 families that followed the localisation of \textit{BRCA1} to chromosome 17q in 1990 (31). This showed that only about half of families with breast cancer only could be attributed to \textit{BRCA1} although most families with breast and ovarian cancer were due to this gene. Additional evidence supporting the existence of another gene came from study of families with at least one case of male breast cancer (and usually several of female breast cancer and/or ovarian cancer) in which strong evidence against linkage to \textit{BRCA1} was obtained (32). This indicated that another gene almost certainly existed and that it conferred a greater risk of cancer in male carriers than \textit{BRCA1}.
A genomic linkage search ultimately revealed the location of \textit{BRCA2} within a 6 CM interval on chromosome 13q12–13 (33). Using a positional cloning approach \textit{BRCA2} was identified towards the end of 1995 (34). Although employing conventional strategies and technologies, the identification of \textit{BRCA2} was notable for the contribution made by sequencing of the 1 mega-base interval within which the gene, by that stage, was believed to reside (35).

**Structure of \textit{BRCA2}**

The \textit{BRCA2} gene is composed of 27 exons (36), of which the first is noncoding. An unusual structural feature is the presence of a large exon 11 of more than 5 kb. Of interest, but of unknown significance, is the fact that \textit{BRCA1} also has an unusually large exon 11. The predicted protein of 3418 amino acids does not show strong sequence similarity to any other in the database. \textit{BRCA2} is expressed widely but exhibits high levels of transcript in the testis (36), a pattern that is again similar to \textit{BRCA1}.

There are two structural features that may provide clues to the function of \textit{BRCA2}. First, there is a region very close to the C-terminus of the protein (amino acids 3334–3344) which contains six out of the seven conserved amino acids characteristic of the granin domain. The position of this granin like region is more in keeping with the stereotypical features of other granins than the position of the granin domain in \textit{BRCA1}. However, in common with \textit{BRCA1} but unlike other molecules containing granin sequences, \textit{BRCA2} does not have a hydrophobic signal sequence. The significance of the putative granin domain is therefore yet to be determined.

Second, there are eight copies of a 20–30 amino acid sequence within \textit{BRCA2} (37). The repeats are separated from each other by regions varying from 60 to 300 amino acids. All are within the segment of the protein encoded by exon 11. The sequence of these motifs is not similar to any previously reported and in database searches detects a single predicted protein of unknown function in \textit{C.elegans} which contains only one copy of the motif. To evaluate further the significance of the repeats we have sequenced exon 11 in several mammalian species (unpublished data). Overall, the part of the protein encoded by exon 11 is poorly conserved, with approximately 50% amino acid sequence identity between human and mouse (a level of conservation similar to \textit{BRCA1}). However, some of the motif units are highly conserved, suggesting that they constitute functionally important units. Others, on the other hand, show no evidence of evolutionary conservation and may therefore not serve a function. It would seem that the core sequence was repeated eight times during evolution but that there was redundancy in this event and only some of the repeated motifs are still being used in modern mammals. This hypothesis will, of course, have to be tested directly.

**The risks of cancer conferred by \textit{BRCA2} mutations**

Formal estimates of risks of breast or other cancers in \textit{BRCA2} mutation carriers have not yet been published. In an analysis of two large breast cancer families due to \textit{BRCA2}, the risk of breast cancer was estimated at approximately 70% by age 70, similar to that in \textit{BRCA1} carriers (unpublished data). The ovarian cancer risk is lower than for \textit{BRCA1}, but is elevated over the general population. The breast cancer risk in men is approximately 5% lifetime, 200 fold that of the general population and considerably greater than for \textit{BRCA1}. Other cancers which are probably overrepresented in \textit{BRCA2} mutation carriers include prostate, pancreas, larynx and ocular melanoma. Scrutiny of pedigrees also suggests tentatively that there are excesses of non-Hodgkin lymphoma, stomach cancer, colorectal cancer and sarcoma. All these observations require formal confirmation.

**Mutations in \textit{BRCA2}**

More than 70 distinct germline \textit{BRCA2} mutations have been found and the number is expected to increase further (34,36,38,39 and unpublished data). The pattern of mutations is similar to that observed in \textit{BRCA1} and in many other tumour suppressor genes. At present all clearly disease causing mutations result in premature termination of translation or absence of transcript. Whilst missense mutations may exist it is problematic at present to distinguish them from rare polymorphisms. Approximately 75% truncating mutations are small deletions, 15% small insertions and 10% base substitutions leading directly to termination codons. Occasional mutations that result in splice errors have also been detected. The mutations are distributed throughout the gene without any clear evidence of clustering.

Most mutations have so far been observed only once. A few, however, have been detected on several occasions. 6503delTT has been detected in several British and French families. 6174delT is the main \textit{BRCA2} mutation in Ashkenazi Jews and has been detected in 8% of breast cancer cases, unselected for family history, diagnosed before age 40 (40). If this observation is confirmed, \textit{BRCA2} 6174delT combined with \textit{BRCA1} 185delAG account for 28% of Ashkenazi breast cancer cases diagnosed before age 40. Finally, 999del5 is the main mutation in Icelandic \textit{BRCA2} families (41). Indeed, this \textit{BRCA2} mutation accounts for most highly penetrant familial breast cancer in Iceland, with \textit{BRCA1} making a relatively small contribution. Of male breast cancer cases in Iceland 40% are carriers of \textit{BRCA2} mutations. This high figure is in part attributable to the elevated risk of breast cancer in men carrying \textit{BRCA2} mutations but is also due to the apparently high prevalence of the 999del5 mutation in the Icelandic population (41). In a study of male breast cancer cases ascertained in North America, 14% carried \textit{BRCA2} mutations, a figure which is probably closer to that expected in most populations (38). There appears to be no correlation between position of the mutation in \textit{BRCA2} and risk of male breast cancer.

**Somatic mutations in \textit{BRCA2}**

Loss of heterozygosity (LOH) on chromosome 13q occurs in approximately 90% breast and other cancers from individuals in families linked to \textit{BRCA2} (42,43). It is almost always the wild type allele inherited from the non mutation carrying parent that is lost in the tumours, the pattern expected of a tumour suppressor gene. LOH on chromosome 13q12–13 is detected in 30–40% sporadic breast cancers and in 50–60% sporadic ovarian cancers (44). However, no clearly disease-causing somatic mutations have been observed in sporadic breast or ovarian cancers (45–47), a result similar to that previously reported for \textit{BRCA1}. This similarity in behaviour of \textit{BRCA1} and \textit{BRCA2} is intriguing. It is possible that neither \textit{BRCA1} nor \textit{BRCA2} are involved in the genesis of sporadic breast and ovarian cancer and therefore that the observed allele losses on chromosomes 13q and 17q are directed at other targets. Alternatively one or both genes are
involved but alternative mechanisms of inactivation are operative which obviate the need for somatic mutations.

THE PATHOLOGY OF BREAST CANCERS ARISING IN BRCA1 AND BRCA2 MUTATION CARRIERS

The ability to identify carriers of BRCA1 and BRCA2 mutations has recently prompted studies of the histopathology of breast cancers arising in susceptibility gene carriers. The studies show that cancers arising in BRCA1 carriers are of higher grade, exhibiting more mitoses, more nuclear pleomorphism and less tubule formation than sporadic breast cancers (48,49 and unpublished data). Perhaps as a result of this, a specific subtype of breast cancer characterised by high grade features (medullary carcinoma) is more common amongst BRCA1 mutation carriers than controls. Although, cancers arising in BRCA2 carriers are also of overall higher grade than controls, this is exclusively due to less tubule formation and there appears to be little difference in mitotic rate or nuclear pleomorphism from controls (unpublished data). At present there is no evidence that these histological differences are reflected in higher recurrence rates or mortality, although further studies will have to be carried out to evaluate this issue further (48,49). However, the results indicate that there probably are biological differences between BRCA1 and BRCA2 and the ways in which they predispose to breast cancer.

OTHER BREAST CANCER SUSCEPTIBILITY GENES

Although almost all families with both breast and ovarian cancer appear to be due to BRCA1 and BRCA2, a proportion of families with site specific breast cancer may still be unaccounted for. One plausible candidate gene to account for these is the gene for Cowden syndrome which was recently localised by genetic linkage analysis to chromosome 10q22–q23 (50). Cowden syndrome is characterised by lesions of the skin and mucous membranes, associated with elevated risk of breast cancer, thyroid disease, colonic polyps and developmental abnormalities of the central nervous system.

REFERENCES


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