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BRCA1/2 carriers and endocrine risk modifiers

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Introduction
Genetic research is a rapidly expanding area of oncology. However, genetic changes do not always result in an effect. There is much interest in the interaction of environmental factors with genetic changes resulting in deleterious effects that can cause disease. It is known that some cases of breast cancer arise as a result of a breast cancer predisposition gene and two genes, BRCA1 and BRCA2 (breast cancer gene 1 and breast cancer gene 2) have been characterised. There is much scientific and clinical interest in individuals who carry these predisposition genes in particular, and from the point of view of clinical management a need for investigating the interaction between hormonal factors and BRCA1/2 genetic status.

Genetic predisposition to breast cancer
Cancer is a genetic disease at the cellular level since genetic changes occur during the conversion of a normal cell into a cancer cell. However, in a minority, one of these changes (or a change predisposing to this process) is inherited (in the germline). The penetrance is the chance that such an inherited change will result in disease development.

About 5-10% of breast and ovarian cancers occur as a result of highly penetrant germline mutations in cancer-predisposition genes (Claus et al. 1990, 1991, Easton & Peto 1990). One of these genes, BRCA1, predisposing to breast and/or ovarian cancer, was mapped to the long arm of chromosome 17 in 1990 (Hall et al. 1990, Narod et al. 1991) and cloned in 1994 (Miki et al. 1994). Collaborative studies by the Breast Cancer Linkage Consortium (BCLC) have shown that BRCA1 mutations are responsible for about 50% of families with clear dominant predisposition to breast cancer and over 80% of families segregating both breast and ovarian cancer (Easton et al. 1993, Ford et al. 1998). A majority (32% of the total, Ford et al. 1998) of the remaining high risk breast cancer families, including most families segregating both male and female breast cancer (76% of these), are due to a second predisposition gene, BRCA2, on chromosome 13q12-13, which was cloned in 1995 (Wooster et al. 1995).

BRCA1 and BRCA2
BRCA1 codes for a protein of 1863 amino acids (Miki et al. 1994). Over 100 distinct genetic alterations (mutations) in BRCA1 have been described to date (Shattuck-Eidens et al. 1995, 1997, BIC database, accessible on the internet at http://www.nchgr.nih.gov/dir/lab_transfer/bic/). These mutations are widely scattered across the gene (Fig. 1). A very similar mutation pattern occurs in BRCA2 (Fig. 2) which is about twice as large. These result in a truncation of the BRCA1 protein due to the insertion or deletion of bases in the coding sequence (frameshifts) or nonsense mutations which convert a coding base into a stop codon. This results in a shortened protein product. This is consistent with the hypothesis that BRCA1 acts as a tumour suppressor gene (Smith et al. 1992). Smaller proportions of mutations involve intron/exon splice sites, leading to a truncated protein, or are single amino acid changes (missense mutations). These four types of mutation account for the findings in the large majority of BRCA1 linked families. However, a small proportion of linked families (estimated to be 10-20%) appear to contain no alterations in coding or splice-site recognition sequences. Individuals from some of these families have been shown to express only the wild-type BRCA1 protein (i.e. the copy of BRCA1 linked to the disease is not expressed), suggesting the presence of a regulatory mutation (an alteration in a part of the genome outside the gene which does not code for the BRCA protein, but governs whether the BRCA protein is made or not). Thus, the absence of a BRCA1 mutation in the coding region/splice sites, even after rigorous sequencing, cannot rule out the possibility of involvement of BRCA1.

The carrier frequency of BRCA1 mutations in the general UK population has been estimated indirectly from epidemiological studies at about 1 in 800, with the range of plausible estimates being 1 in 500 to 1 in 2500 (Ford et al. 1995). The corresponding estimated frequencies
amongst breast and ovarian cancer cases at different ages are shown in Table 1. Studies of early onset breast cancer cases in Boston and Seattle have found BRCA1 carrier rates which are in good agreement with these estimates (Fitzgerald et al. 1996, Langston et al. 1996). Much higher frequencies are known to apply to Ashkenazi Jews, owing to a founder effect involving mainly a particular mutation (185delAG; Struwing et al. 1995). This mutation has an estimated frequency of about 1 in 100 in Ashkenazi Jews, and about 1 in 5 in Jewish women diagnosed with breast cancer under 40 (Struwing et al. 1995, Offit et al. 1996). This rises to two-thirds in families with breast and ovarian cancer (Tonin et al. 1996). There is another mutation in BRCA1 which is more commonly seen in Ashkenazis (5382insC) and another in BRCA2 (6174delT) which is present in 1 in 67 of this population (Neuhausen et al. 1996, Oddoux et al. 1996, Roa et al. 1996).

Studies by the Breast Cancer Linkage Consortium have provided estimates of the cancer risks in carriers of mutations in BRCA1 (Easton et al. 1993, 1995, Ford et al. 1994, 1998). Based on the incidence of disease in linked families, Easton et al. (1995) estimated the risk of breast cancer to be 51% by age 50 and 85% by age 70. The risk of ovarian cancer has been estimated to be 63% by age 70, based on the ovarian cancer incidence in linked families or 44% based on the risk of ovarian cancer in carriers with a previous breast cancer (Easton et al. 1995). There is some evidence that the ovarian cancer risk is mutation

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**Figure 1** BRCA1 mutation spectrum (from BIC database). Exon 11 was arbitrarily divided because of its large size.

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**Figure 2** BRCA2 mutation spectrum (from BIC database). Exon 11 was arbitrarily divided because of its large size.
dependent; Gayther et al. (1995) found that mutations towards the 5' end (the beginning) of BRCA1 confer a higher risk of ovarian cancer than those towards the 3' end, a result also found by Holt et al. (1996). At present, this evidence for allelic heterogeneity (the fact that different mutations may have different risks) is not used in counselling but if the evidence becomes more reliable, this may change. In general, most cancer geneticists quote a lifetime ovarian cancer risk from a BRCA1 mutation of 40-60%. The risk of ovarian cancer in BRCA2 is also raised (27% by age 80) but not as high as for BRCA1 (Ford et al. 1998) (see Figs 3 and 4).

Function of BRCA1 and BRCA2

The function of the BRCA1 and BRCA2 proteins is a topic of intense research activity at present. Preliminary data suggest that the BRCA1 and BRCA2 proteins form part of a complex at the sites of DNA damage. They complex with Rad51 and Rad52 which are homologous to DNA repair proteins in E. coli (Kastan et al. 1991, Scully et al. 1997). BRCA1 and BRCA2 are thought to be ‘caretaker’ genes, responsible for maintaining genome stability (Kinzler & Vogelstein 1997, Shen et al. 1998, Xu & Morris 1999). When BRCA1/2 are faulty or absent, the accumulation of unrepaired, damaged DNA results in tumour formation.

<table>
<thead>
<tr>
<th>Age</th>
<th>Breast (%)</th>
<th>Ovary (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>7.5</td>
<td>5.9</td>
</tr>
<tr>
<td>30-39</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>40-49</td>
<td>2.2</td>
<td>4.6</td>
</tr>
<tr>
<td>50-59</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>60-69</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>20-69</td>
<td>1.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

From Ford et al. (1995).

Table 1 Proportions of single cases due to BRCA1 by age (estimated from the gene frequency)

<table>
<thead>
<tr>
<th>Chance (%) that mutation is present†</th>
<th>Clinical criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>All single cases of breast or ovarian cancer</td>
</tr>
<tr>
<td>10</td>
<td>Single breast cancer cases &lt; 35 years</td>
</tr>
<tr>
<td>≥20</td>
<td>≥2 breast cancer cases &lt; 50 years</td>
</tr>
<tr>
<td>≥20 ≤50</td>
<td>1 breast cancer &lt; 40 years in an Ashkenazi</td>
</tr>
<tr>
<td>&gt;50</td>
<td>&gt;1 breast and ovarian cancer</td>
</tr>
<tr>
<td></td>
<td>≥4 cases of female/male breast cancer</td>
</tr>
<tr>
<td></td>
<td>6+ female breast cancers</td>
</tr>
</tbody>
</table>

†NB The chance of detecting a mutation is lower because: (1) at least 15% of mutations are regulatory, i.e. are not in the coding region of the gene which is the area tested, and (2) the genetic screening methods are about 80% sensitive.

From: Langston et al. (1996), Shattuck-Eidens et al. (1997) and Ford et al. (1998).

Figure 3 Breast (●) and ovarian (■) cancer risks in female carriers of BRCA1 mutation.
Genetic testing for BRCA1/2

Since mutations can be widespread throughout the BRCA1 and BRCA2 genes, when offering genetic testing to a family, the first step in testing is to identify the specific mutation pertaining to the family being counselled. The chance of a BRCA1/2 mutation being present is shown in Table 2. This involves taking blood from a live affected member of the family, since these individuals are more likely to harbour a breast cancer predisposition gene. Mutation screening is then performed on DNA from the blood sample to ascertain which specific mutation is present in the family. An unaffected relative is only offered mutation testing for the specific mutation, previously found in their affected relative. This is accompanied by full counselling, with at least two counselling sessions 1 month apart (the so-called ‘cooling off’ period). The presence of a negative genetic test in this situation is truly negative, since the mutation has already been identified in the family. When a mutation is not identified in the initial mutation screen, this does not exclude that a gene is present, as discussed earlier. The uptake of predictive genetic testing is higher for breast cancer families than in other genetic diseases, where preventative measures cannot be offered (e.g. Huntington’s disease where the uptake of testing is about 16%; Craufurd et al. 1989). In research families, the uptake of BRCA1 testing is about 44% overall and is higher in women than in men (Watson et al. 1995, 1996). At 1 year post-test result, Watson et al. (1996) did not show any adverse psychological features.

Individuals from the general population found to carry BRCA1/2 mutations may have a much lower breast cancer risk than those who are members of families with multiple cases of breast cancer (37-50%) (Strueming et al. 1997, Thorlacius et al. 1998). The variability in penetrance in various populations suggests a role for other factors, genetic or environmental, in cancer development in BRCA1/2 carriers. There is also variability in clinical presentation; a woman carrying a BRCA1 or BRCA2 mutation can live to the age of 80 without cancer, while others present with breast cancer in their 20s. Many environmental factors such as diet, exercise, carcinogen exposure and unknown external factors could be responsible for these differences. Hormonal factors are now being studied.

Endocrine factors and BRCA1/2 genetic status

The role of endocrine factors in breast and ovarian cancer has been extensively investigated in the general population. Exposure to oestrogen is known to elevate the risk for breast cancer. Early menarche and late menopause increase the risk for breast cancer (Henderson et al. 1993). Oral contraceptive (Collaborative Group on Hormonal Factors in Breast Cancer 1996) and hormone replacement therapy (Beral et al. 1998) use is associated with a small increase in the risk for breast cancer which is related to the length of use, and time lapse from period of use. Ovarian
in hormonal factors are involved in malignant transformation; early menarche and late menopause increase the risk, whereas multiple pregnancies and use of oral contraceptives lower the risk. Whether endogenous and exogenous hormonal factors are involved in malignant transformation in BRCA1/2 carriers is a question of great importance since one method of lowering the cancer risk in carriers could be based on hormonal chemoprevention.

The interaction of hormones with the BRCA1/2 genes has been studied in cell lines and in mutation carriers. In breast cancer cell lines, the BRCA1 mRNA and protein were increased by sex steroids (Gudas et al. 1995). The expression of BRCA1 mRNA in murine mammary gland was elevated during puberty, pregnancy and following treatment with oestradiol and progesterone, suggesting a role for the BRCA1 gene in the process of proliferation and differentiation in response to ovarian hormones (Marquis et al. 1995). Thus in the presence of a mutated gene, hormonal stimuli theoretically cause proliferation of transformed cells.

Few attempts have been made to investigate the risk conferred by various hormonal factors in human BRCA1/2 mutation carriers. These studies have many biases and their results therefore should be interpreted with caution. Most studies only include living carriers which, by virtue of the fact that they have to be alive to have a genetic test, bias results towards an improved survival. Another problem with these studies is the control group, which ideally should be composed of healthy carriers with the same age structure as the affected group. However, since the risk for cancer development is very high amongst BRCA1/2 mutation carriers, it is often difficult to find a large enough control group which is unaffected. Even with these reservations, more data are accumulating which suggest a modifying effect of hormonal factors in BRCA1/2 gene carriers.

In a historical cohort (Narod et al. 1995) the risk of breast cancer in BRCA1 carriers was found to decline with increasing parity, in the same way as in the general population. However, young age at first pregnancy did not confer additional protection. Interestingly, the factor most significantly associated with the risk of breast cancer was year of birth after 1930. It could be that different demographic factors, as yet unknown, elevate the risk for breast cancer in carriers.

Table 3 Lifetime (by age 80) cancer risks (%) in BRCA1/2 carriers.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female breast</td>
<td>80–85</td>
<td>80–85</td>
</tr>
<tr>
<td>Ovarian</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>Male breast</td>
<td>70</td>
<td>?0</td>
</tr>
<tr>
<td>Colon</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Prostate†</td>
<td>6</td>
<td>6–14</td>
</tr>
</tbody>
</table>

Table 3. Lifetime (by age 80) cancer risks (%) in BRCA1/2 carriers.

Two important studies have recently been published. The first studied the effect of oral contraceptive use on the risk of ovarian cancer in BRCA1/2 carriers (Narod et al. 1998). The Pill is known to lower the risk of ovarian cancer in the general population and was found to have the same effect in carriers. The odds ratio for ovarian cancer for BRCA1 carriers who had used oral contraceptives was 0.5, and that for BRCA2 carriers was 0.4. The risk of ovarian cancer decreased with longer duration of Pill use. The risk of breast cancer conferred by the use of oral contraceptives in carriers was not tested. This study is a good example of the limitation of epidemiological studies conducted in this population. The control group in this study was composed of 161 living sisters who were not diagnosed with ovarian cancer. Among them only 95 were tested for the mutation found in their family. Forty-two were non-carriers. Furthermore, 67 of the control group underwent bilateral oophorectomy at an average age of 45. However, since prospective randomised trials are hard to conduct and will take a long time to provide prospective data, counselling of carriers has to be based on such retrospective studies, taking into account the limitations of the data. Another small study conducted among Ashkenazi breast cancer patients found higher long-term oral contraceptive use before first full-term pregnancy in carriers than in non-carriers. This may suggest that the use of oral contraceptives might increase the risk for breast cancer in BRCA1/2 carriers more than in non-carriers (Ursin et al. 1997).

Other lifestyle factors

A provoking article, recently published, reported the influence of smoking on the risk of breast cancer in BRCA1/2 carriers (Brunet et al. 1998). They found that carriers who had smoked more than 4 pack years had an odds ratio of 0.46 compared with carriers who never smoked. The authors suggested that the effect of smoking in BRCA1/2 carriers could be mediated through hormonal pathways. Cigarette smoke has been associated with early menopause, an increased risk of osteoporosis and a decreased risk of endometrial cancer. There is no evidence that cigarette smoking is directly anti-oestrogenic but altered oestrogen metabolism has been demonstrated in...
female smokers in whom the formation of inactive 2-
hydroxy-oestradiol derivatives is enhanced (Michnovicz
et al. 1986) which might have a protective role in BRCA1/
2 carriers. Obviously, the other carcinogenic effects of
smoking preclude its use as a preventative strategy, but the
underlying mechanism provides supportive evidence that
anti-oestrogenic chemopreventative manoeuvres could be
effective in BRCA1/2 carriers. The interaction of other
lifestyle factors with BRCA1/2 status (e.g. diet) is
unknown.

Chemoprevention

Three recent chemoprevention trials have reported
conflicting results (Fisher et al. 1998, Powles et al. 1998,
Veronesi et al. 1998). The NCI-NSABP-P1 study showed
a 45% reduction in the incidence of breast cancer in the
group of women taking Tamoxifen versus those taking
placebo. However, this reduction was seen very early in
the study (at 2 years), sooner than a preventative agent
would have been expected to act, and Powles’ and
Veronesi’s studies did not show this. This may be due to a
treatment effect of occult breast cancers in the NSABP-P1
study. latter study recruited women at a relatively low
breast cancer risk but Powles’ study had a larger
proportion of women who were likely to have a breast
cancer predisposition gene. Genetic screening in
chemoprevention studies will be very important to
determine if there is an interaction between efficacy and
 genetic status.

The Breast Cancer Linkage Consortium was originally
established to perform genetic localisation studies. This is
a large collaborative group of individuals interested in
breast cancer genetics which combines data from families
with alterations in breast cancer predisposition genes.
There are currently large studies, in collaboration with this
group, to determine whether lifestyle and hormonal
factors interact with BRCA1/2 genetic status. It will be
important to continue to combine data from the relatively
small number of individuals with germline mutations in
BRCA1/2, as the level of the interactive effect between
hormonal factors and genetic status is unknown.

Conclusions

The conclusions of this review can be summarised as
follows:

Only 5-10% of breast cancers occur in individuals
with a high risk breast cancer predisposition gene.
Of these 5-10%, about half are due to BRCA1/2, so
further genes remain to be discovered.
Due to founder effects, the Ashkenazi population has
a higher chance of having specific mutations.

There is some evidence that endocrine factors may
modify penetrance.

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