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Feature Article

The Genetics of Familial Breast Cancer and Their Practical Implications

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A small proportion of breast cancer (perhaps about 5%) and a higher proportion of early onset cases are due to the inheritance of mutations in dominant susceptibility genes which confer a high lifetime risk of the disease. This would equate to about 1250 cases per year in the U.K. and 9000 in the U.S.A. Even within these cases, there is genetic heterogeneity, i.e. there are several genes involved, each giving rise to different patterns of other cancers associated with the familial breast cancer. One such gene (p53) has been identified and a second (BRCA1) has been precisely mapped in the human genome, but further breast cancer predisposition genes remain to be identified. In addition, there are other genes which confer a lower risk of the disease, but may account for a larger proportion of cases, the most important example to date being ataxia telangiectasia. The identification of these genes will enable the entity of familial breast cancer to be more precisely defined and has implications for management of gene carriers with breast cancer and their relatives who are at risk. A major consideration in this new area of cancer genetics is that the identification of gene carriers may become possible on a large scale and this raises ethical and social issues.

Key words: familial breast cancer, genes, implications
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INTRODUCTION

OVER THE past few years, there has been a dramatic improvement in our understanding of genetic predisposition to cancer. Although an inherited component to human cancer has been recognised for many decades, the evidence for genetic predisposition to cancer has historically been based on anecdotal reports of families with unusually high numbers of cancer cases apparently consistent with Mendelian inheritance, the cancers often being of types rare in the general population (the so-called Mendelian cancer syndromes), together with more systematic epidemiological evidence indicating that most cancers are significantly more common in close relatives of cancer patients with the same cancer type. Over the past decade, the genes for many of the clearly inherited cancer syndromes have been localised to chromosomal regions, usually by genetic linkage studies. More significantly, a number of genes have been localised which are responsible for familial clustering of the more common cancers such as breast, ovarian and colon cancer, for which previously the evidence of an inherited component was more equivocal.

The aim of the human genome project is to clone the majority of the expressed human genome by the next century. Linkage analysis is used to study the inheritance of genetic markers that are close to the chromosomal locations of disease-predisposition genes and are therefore co-inherited. The rapid progress in this field is principally due to a number of important technical advances, notably the very large number of polymorphic genetic markers throughout the human genome which can be used for linkage analysis [1] and the polymerase chain reaction which has greatly increased the speed of analysis and has enabled DNA in paraffin-embedded tissue from deceased family members to be studied. This has substantially increased the numbers of families large enough for linkage analysis.

The cloning of genes has made their direct testing as candidates for causing disease possible, without the study of large families by linkage. As more genes are cloned in the human genome project, the direct testing of genes as candidates for diseases will increase. It will also be possible, by direct gene analysis, to detect genes causing more moderate risks (which could potentially be reponsible for a higher proportion of cancer cases), which are more difficult to detect by linkage analysis, since these are less likely to give rise to dramatic families with large numbers of cases.

The identification of these susceptibility genes is providing

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important insights into the mechanisms of carcinogenesis since many of them have proved to be involved, not only in inherited cancers (through germline mutations), but also in a high proportion of "sporadic" cancers, where the same gene is mutated somatically. However, the more immediate practical consequence of these discoveries is that individuals at a greatly increased risk of particular cancers can be identified. Determining the appropriate management of these individuals is proving a major challenge for oncologists and geneticists.

Breast cancer has been recognised as having a familial component for many years [2]. Several breast cancer predisposition genes have already been cloned, but their contribution to familial breast cancer is small. The discovery that a gene predisposing to breast cancer in certain families was located on the long arm of chromosome 17 (17q; [3]) has generated a high level of anticipation in the scientific and medical community and amongst women in breast cancer families. It is expected that this gene will be cloned soon; once this is achieved, its precise contribution to overall breast cancer incidence can be determined.

THE SIZE OF THE PROBLEM

It has been estimated that about 5% of breast cancer may be due to dominant cancer predisposition genes [4]. In the U.K., this would equate to 1250 incident cases per year and to 9000 in the U.S.A. Approximately 1 in 200 women will develop breast cancer in the western world as a result of genetic predisposition. The average U.K. general practitioner's list will, therefore, include 5–10 such women, and a district hospital breast clinic will expect 10–20 new cases of breast cancer attributable to genetic predisposition per year.

It is important to identify the genes involved for several reasons; (a) the knowledge about the mechanisms of action of these genes will direct new approaches to the treatment and prevention of the disease in general, (b) the identification of carriers will enable targeted prevention of those at higher risk, (c) some genes may predispose to cancers other than breast cancer, and this may alter the management of breast cancer in gene carriers, and require prevention of cancers other than breast cancer in at-risk relatives.

There are several possible genetic models which might explain the observed familial clustering of breast cancer. One is a rare, highly penetrant autosomal dominant gene which conveys a high risk of breast cancer to those who are gene carriers, accounting for a small percentage of breast cancer cases. There is evidence from segregation analyses of collections of families that at least one such gene exists. Analysis of families in a study of Cancer and Steroid Hormones in America (CASH) reports evidence for an autosomal dominant gene with a gene frequency of 0.0033 [4]. This hypothesised susceptibility gene has an estimated penetrance for breast cancer of 82% lifetime, and it was predicted that inherited susceptibility affected 4–5% of the families studied.

The true situation in the general population is probably a mixture of the occurrence of high-risk, rarer breast cancer predisposition genes, such as BRCA1, and more common genes which convey a lower breast cancer risk in those who are gene carriers. Since the latter genes could be common, they could contribute to a higher percentage of breast cancer cases and, therefore, be a more significant public health problem. If this were the case, genetic predisposition to breast cancer could contribute to a higher proportion of all breast cancer cases than 5%.

Clinically, the higher risk genes manifest as families with clustering of breast cancer and in some instances, other cancers. Until recently, these families were described phenotypically depending on the types of cancer present, but with the discovery of the genes responsible, in the future, they will be described genetically. Genes conferring a lower risk to the individual will not cause clustering of cases and could be misinterpreted as sporadic cases. Only the cloning of candidate genes and testing for them in apparently sporadic cases will determine their contribution to the total breast cancer burden.

WHEN IS BREAST CANCER FAMILIAL?

Since breast cancer is the commonest female cancer in western countries, the probability of a woman having one first degree relative with breast cancer is high due to chance alone. Epidemiological studies have shown that the chance of a breast cancer-predisposing gene being present in a family increases as the number affected increases, the age of the affected decreases [5] and if there is recent bilateral disease [6, 7].

The genetic models constructed using data from the CASH study [5] have suggested that even if a single affected case is young at diagnosis, that individual still has, at most a 36% chance of being a gene carrier if affected at age 20–29 years. However, if two first degree relatives (a mother and sister or two sisters) are affected at age 30, the chance of a gene being present in the family rises dramatically to 90% (Figure 1, courtesy of Prof. D.T. Bishop).

Some breast cancer predisposition genes also predispose to other cancers. These types of cancer indicate which gene may be involved. For example, the gene p53 not only predisposes to early onset breast cancer, but also sarcoma, brain tumours, leukaemia and adrenocortical carcinoma (the Li–Fraumeni syndrome).

THE GENETIC HETEROGENEITY OF THE DISEASE

Several of the genes causing inherited predisposition to breast cancer have now been located. They vary widely in the risks of breast cancer they confer, their overall contribution to breast cancer incidence, and to the familial clustering of breast cancer and spectrum of other cancers (Table 1).

The genes for which an increased risk of breast cancer in carriers is firmly established are the BRCA1, p53 and androgen receptor genes. There is also some suggestion that breast cancer risk is elevated in carriers of hereditary non-polyposis colon cancer genes, of which two have now been isolated [9–12], and that carriers of the ataxia telangiectasia gene and certain HRAS alleles have a higher breast cancer risk than the general population (Table 1). These do not account for all breast cancer families,and therefore further genes remain to be located. If the

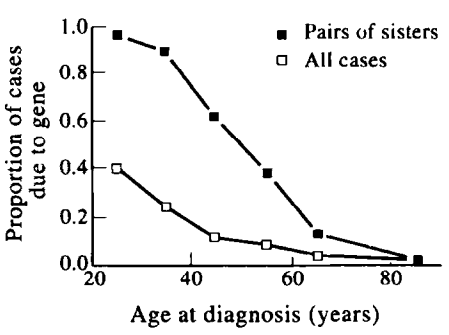

Figure 1. The proportion of breast cancer cases due to autosomal dominant predisposition genes by age of the case.

*Table 1. Genes predisposing to breast cancer**

Gene	Chromosomal location	Breast cancer risk by age 50 years	Breast cancer risk by age 70 years	Risk of any cancer by age 50 years	Risk of any cancer by age 70 years	Proportion of breast cancers due to gene by age <50 years	Proportion of breast cancers due to gene by age 50+ years	Associated cancers
p53	17p	55%	?	80%	?	<1%	–	Sarcomas, brain, adrenocortical cancer, leukaemia
BRCA1	17q	49%	71%	58%	85%	5%	1%	Ovary Colon? Prostate?
BRCA2	?	?	?	?	?	?	?	Male breast, prostate?
AT	11q	11%	30%	15%	46%	7%	7%	Lymphoma, leukaemia
HRAS	11p	3%	10%	9%	10%	—	79%	All cancers?
AR	Xq	??	(Male BC only)			<<1%		

*Modified from Easton *et al.* [18]. BC, breast cancer.

lower risk but more prevalent gene model is correct, it is possible that the most important breast cancer predisposition gene, with respect to its impact upon the entire population, may still await identification.

THE CURRENT STATUS OF THE IDENTIFICATION OF AND EFFECTS OF BREAST CANCER PREDISPOSITION GENES

BRCA1

In 1990, Hall and colleagues described linkage to a locus on chromosome 17q in seven breast cancer families with early onset breast cancer [3]. This linkage was confirmed by Narod and colleagues [13] in families, segregating both breast and ovarian cancer, and subsequently by The Breast Cancer Linkage Consortium (BCLC), a collaboration of 13 international groups, in a series of 57 breast/ovarian cancer families and 157 breast cancer families without ovarian cancer [14]. In the BCLC analyses, the estimated proportion of breast/ovarian cancer families linked to BRCA1 was 100%, whereas only 45% of the breast-only families were linked.

The location of BRCA1 has been narrowed to a region of less than 1.5 Mb [15, 16]. There is some uncertainty about the overall contribution of this gene to familial breast cancer. Initial reports suggested that all early onset breast cancer families might be due to BRCA1 [3], but subsequent evidence from the overview of linkage results worldwide from the BCLC suggest that <50% of the breast-only families are linked [14]. It also appears that a proportion of families segregating both breast and ovarian cancer and the majority of families, which include both male as well as female breast cancer, are not linked to BRCA1 [17].

Estimates of the penetrance of the BRCA1 gene have been made from the BCLC data [14] and from large linked kindreds [18]. These estimate the risk of breast cancer in carriers to be 49% by the age of 50 years, and 71% by age 70, and the risk of ovarian cancer to be about 42% by age 70 (Easton *et al.*, submitted). There is, however, some suggestion of variation in cancer risk between different BRCA1 families, particularly for ovarian cancer. Some families would appear to have a lifetime risk of ovarian cancer close to the breast cancer risk, whereas other families have an ovarian cancer risk which is more moderate.

There is also evidence that BRCA1 mutation carriers have a 3-fold increased risk of prostate cancer and 4-fold increased risk of colon cancer [19], although the absolute risks of these cancers are small in comparison with the breast and ovarian cancer risks, and they need to be confirmed in further studies.

Other genes

p53. Early onset breast cancer occurs as an important component of the Li–Fraumeni syndrome, a dominant cancer syndrome in which gene carriers have a high risk of childhood sarcomas, early onset breast cancer, brain tumours, leukaemia and adrenocortical carcinoma [20]. At least 50% of Li–Fraumeni families have germline mutations in the oncogene p53 [21]. Several groups have looked for germline p53 mutations in breast cancer families which are not classical Li–Fraumeni families [22–23]. Of the 87 families with early onset breast cancer reported in the literature, four have p53 germline mutations and all but one have features of Li–Fraumeni families. Further systematic studies have shown that germline p53 mutations account for much less than 1% of all breast cancer cases [24, 25], and this is mainly in the context of the Li–Fraumeni syndrome or Li–Fraumeni-like families.

hMSH2 and hMLH1

Hereditary non-polyposis coli (Lynch type II or HNPCC) families consist of non-polyposis colon cancer in young adults and a variety of malignancies including pelvic, gastric and skin cancer. These families can also have individuals with sarcomas and brain tumours and some workers report an increased incidence of breast cancer (relative risk of breast cancer of 5; [9]). In several families with this structure, linkage to chromosomes 2p and 3p has been reported and the genes responsible are the human homologue of bacterial and yeast mut L&S genes; hMSH2 [10, 11] and hMLH1 [12], both involved in repair of mismatched DNA bases. However, the absolute risk of breast cancer appears to be low in carriers of mutations in these genes.

Androgen receptor (AR)

Mutations in the AR gene on the X chromosome have been described in two families with *male* breast cancer and incomplete androgenisation [26, 27]. To date, no germline mutations in the AR gene have been found in female breast cancer, and the

proportion of male breast cancer cases due to germline AR mutations also appears to be low. Furthermore, many families with multiple cases of male breast cancer have been shown not to be due to the AR gene because of the observation of male/male transmission of the disease.

HRAS

Certain alleles in a minisatellite region of the HRAS gene have been shown to be associated with an approximate doubling of the risk of most common cancers, including breast cancer [28]. This remarkable observation has as yet no obvious explanation. Nevertheless, given the prevalence of these mutations, about 9% of breast cancer could be attributable to this susceptibility gene.

Ataxia telangiectasia (AT)

AT is a rare disease caused by an autosomal recessive gene; homozygotes are characterised by ataxia, telangiectases, immunological deficiency and neoplasia [29]. AT heterozygotes have a substantially increased risk of developing breast cancer with an estimated relative risk of about 7-fold [30], and some increased risk of other cancers. Although this risk is much lower than that conferred by p53 or BRCA1, AT mutations could account for a higher proportion of incident breast cancer cases than either of these genes because AT mutations are more common. The population frequency of AT heterozygotes is estimated to be about 1.4% [31], and on this basis, AT could account for about 7.5% of all breast cancer cases. This compares with an estimated 2% for BRCA1 and less than 1% for p53 (Table 1). Given the moderate risk conferred by AT, it does not account for a significant fraction of breast cancer families, as has been confirmed by linkage analyses [32].

Of the genes so far identified, only BRCA1 is likely to contribute significantly to the observed familial clustering of breast cancer. p53 mutations confer a high risk, but are too rare to account for more than a small fraction of cases. AT heteroxygotes and the rare HRAS alleles are more common, but confer a moderate breast cancer risk.

Other genes

There has been a suggestion of linkage in one family with older onset female breast cancer to the oestrogen receptor locus on 6q [33], but this has not yet been replicated.

A few high-risk families, clearly associated with a dominant gene but which are not due to any of the genes described in this article have been identified, and these genes are yet to be discovered [17].

THE IMPLICATIONS OF PREDICTIVE GENETIC TESTING FOR INDIVIDUALS IN BREAST CANCER FAMILIES

Management implications of those already affected

The possibility that an individual has developed breast cancer as a result of genetic predisposition may have implications for their treatment. The most obvious example of this is that patients with a strong family history are more likely to develop contralateral breast cancer. This has been shown most dramatically in those individuals carrying BRCA1 who have a risk of 87% by age 70 of contralateral breast cancer (Easton *et al.*, submitted). A particularly careful assessment should, therefore, be made of the other breast. Anecdotally, familial breast cancer is also associated with multicentricity [34]. Some oncologists, particularly in the U.S.A. would consider mastectomy in preference to a conservative approach, even for early disease in a BRCA1 carrier.

The patient may be at risk of cancers at other sites, for example, a BRCA1 carrier from a breast/ovarian family, who has breast cancer, would also be at a lifetime risk of over 50% for ovarian cancer, and oncologists may, therefore, consider prophylactic oophorectomy for such patients. The latter would not only be a preventative measure for ovarian cancer, but is also known to improve the recurrence rate and survival from breast cancer (Early Breast Cancer Trialists' Collaborative Group, [35]). Although this sounds like a drastic management approach, several oncologists in the U.S.A. are anticipating testing all their patients with breast cancer for the BRCA1 gene once it is cloned, and would then offer them at least bilateral mastectomy as part of their management (Skolnick, personal communication). The approach in the U.K. will almost certainly be more conservative until the disease parameters in gene carriers have been more thoroughly studied.

It is not known whether gene carriers with breast cancer have a different prognosis. Some studies have suggested that familial breast cancer as a whole has a better prognosis [36–38]. However, the problem with these studies is that they contain potential bias because relatives of breast cancer cases are more likely to seek earlier management for breast symptoms. Although correction was made for stage at diagnosis in these studies, there could still be a hidden bias in the results.

There is evidence that lobular carcinoma *in situ* [39] and lobular invasive breast cancer [40] are more often associated with a positive family history, but otherwise, familial breast cancer seems to have the same histological features as sporadic forms of the disease.

Gene testing could also influence management if the presence of the cancer predisposition gene altered the patient's response to certain types of therapy. Fibroblasts from Li–Fraumeni patients have increased transforming ability and reduced radiosensitivity in culture [41, 42]. The problem with such experiments is that the p53 mutation was not correlated with radiation response because these experiments were performed before the p53 gene was cloned. However, mouse p53 heterozygotes also show intermediate radioresistance between normal and p53 homozygotes [43]. If these experiments can be extrapolated to man (an assumption), irradiation of p53 heterozygotes could conceivably increase the risk of second malignancy because radiation damage will be tolerated by the irradiated cells which would not be lethally damaged. Mastectomy may, therefore, be a better management of breast cancer in these patients than conservative surgery and irradiation. The effect of chemotherapy in these individuals is unknown.

There is some concern that AT heterozygotes will be more radiation sensitive than normals because AT homozygotes are exquisitely radiation sensitive [44]. There is controversy about this [45], but if this were the case, gene testing for AT could become very important. There would also be concern over the use of screening mammography in known AT heterozygotes if the risks from irradiation were shown to be high.

It is not known whether BRCA1 carriers have a different response to radiation and chemotherapy.

Management implications for at-risk relatives

The possibility that an individual is affected with familial breast cancer not only has implications for their own treatment, but also raises the possibility that their blood relatives may be at risk of the disease. The effect of identifying genetic breast cancer, therefore, extends to the whole family. There are a number of early detection and preventative options for such

individuals, many are unproven and so should ideally be offered within research protocols.

Screening. Gene carriers in families can be offered targeted screening for tumours to which the gene predisposes, although in many cases, the effect of screening is unproven. Screening for breast cancer by mammography is a superficially attractive option since it is already widely used and is non-invasive. There is clear evidence that it can reduce breast cancer mortality in women over 50 years [46]. Three-yearly mammography as used in the U.K. national screening programme reduces mortality by about 30%. Under the age of 50 years, mammography has a lower impact on mortality. A recent meta-analysis of randomised trials estimated a mortality reduction in the under 50s of 10% (95% CI 35% mortality reduction up to a 24% detrimental effect; [47]), and some would argue that mammography under 50 years has no proven benefit. However, even if the benefit were a 10% reduction in mortality, the overall mortality from breast cancer by age 70 in, for example, a BRCA1 carrier, would only be reduced from about 35 to 31%. Despite this, screening by mammography of high-risk women is widespread (typically annually from age 35), and so needs to be properly studied. To this effect, a national U.K. register through the screening centres has been proposed for high-risk women screened by mammography (Cuckle, personal communication). A randomised trial would be preferable, but would be difficult to effect because many women at increased risk would not accept a no-screening arm in a trial.

The position with regard to ovarian cancer screening in breast/ovarian cancer families is even less satisfactory. Ovarian screening by annual transvaginal ultrasound is commonly offered to women with a family history of ovarian cancer, particularly where two members of a family are affected, but it is not known whether this has any impact on mortality [48].

Prophylactic surgery. The most drastic treatment option available to high-risk individuals is removal of the organs at risk since different cancer-predisposition genes seem to be tissue-specific. In particular, prophylactic removal of the ovarian and breast tissue can be offered to BRCA1 carriers. Although it would appear self evident that this would substantially reduce the cancer risk, there is no systematic evidence that this is the case.

When considering prophylactic mastectomy, the amount of tissue removed depends on the operative technique; subcutaneous mastectomy is avoided by many surgeons because 10–15% of breast tissue remains [49]. There have been several reported instances of subsequent breast cancer following subcutaneous prophylactic mastectomy, but its exact incidence is unknown [50].

Prophylactic oophorectomy has been followed in a number of cases by peritoneal adenocarcinoma, histologically identical to ovarian carcinoma and close examination of the resected ovaries has failed to reveal primary ovarian cancer. It is thought that the cells lining the peritoneal cavity which would carry the same mutation, are also susceptible to the development of ovarian cancer. The incidence of this occurrence after oophorectomy is unknown, but Tobacman and colleagues [51] reported three such cancers (11%) among 28 women in high-risk families who had prophylactic oophorectomies. Even if this failure rate is exaggerated, it nevertheless indicates that better prophylactic measures are needed.

The precise degree of protection offered by prophylactic oophorectomy and mastectomy is difficult to assess because the studies cannot estimate the cancer incidence which would have occurred if the operation had not been done. All that is certain is that these operations do not provide 100% protection from development of the relevant cancers.

Chemoprevention. Chemoprevention of cancer is gene carriers provides an exciting alternative to prophylactic surgery for preventing cancer in high risk groups. If this approach were effective, the identification of gene carriers would justify the use of chemopreventive agents, which have potential side-effects. In breast cancer, the agent at the most advanced stage of testing for chemoprevention is tamoxifen. It reduces the risk of a second primary breast cancer in those who have already had one primary [52], and it has been piloted as a chemopreventative agent in a trial of 1700 women at increased risk of breast cancer because of a positive family history [53]; these cancer prevention results have yet to mature. Larger trials to assess its efficacy are underway. Although the toxicity profile of tamoxifen in this pilot study was low, some workers have raised objections to its wider use in the general population to prevent breast cancer because of its rare side-effects (an increased risk of endometrial cancer in humans and liver cancer in animals; [54]; and its ocular toxicity; [55]). Nevertheless, the use of tamoxifen might still be justified in high-risk individuals where the absolute benefits would be greater. Its risk of endometrial carcinoma would make it an unacceptable drug in Lynch type II families where there is already a 4-fold risk of this disease. Unfortunately, tamoxifen may not be useful for many BRCA1 carriers because it cannot be given to women under 35 years (preferably not under 45 years), because of its teratogenicity. A large proportion of the breast cancer risk will therefore remain, because the risk of breast cancer is substantial in such gene carriers, even at young ages (20% by age 40); in addition, it is not thought to prevent ovarian cancer. The development of an oral contraceptive pill based on suppression of ovulation using a GnRH agonist which aims to prevent both breast and ovarian cancer would be an exciting new development for young carriers of the BRCA1 gene [56].

Gene–environment interactions. Lifestyle factors may influence the penetrance of the gene in carriers. An important issue is the impact of the combined oral contraceptive pill on cancer risk in high-risk women. There is evidence [57] that breast cancer risks at young ages are increased in long-term pill users, but conversely, the ovarian cancer risk is reduced. The overall effect on cancer risk in BRCA1 carriers is difficult to predict, because it is unknown whether the pill will have the same quantitative effect in gene carriers as in the general population. However, assuming that this is so, there would be a substantial increase in the overall cancer risk by age 40 years, and the protective effect on ovarian cancer would have little effect since the genetic risk is much less at this age. However, lifelong cancer mortality may be reduced because risk of breast cancer occurs predominantly at younger ages, whereas the ovarian cancer risk is long-term and has a poorer prognosis. Assuming a 50% lifelong protection against ovarian cancer, and a 1.8-fold increased risk in breast cancer by age 45, the risk of either cancer by age 45 is increased from 45 to 59%; however, the risk of cancer by age 70 is unchanged by taking the combined pill. Nevertheless, cancer mortality would be reduced because the mortality from ovarian cancer is higher. These calculations may not apply to all BRCA1 families, particularly those with a lower ovarian cancer risk, and are subject to considerable uncertainty, which will be reduced when the gene is cloned and large epidemiological studies of gene carriers are performed.

Similar arguments in terms of breast cancer risk exist for hormone replacement therapy, although the risks are acting at older ages when, if breast cancer has not developed, the individuals are less likely to be carrying the gene. When predictive genetic testing is available and gene carriers can be identified, this will enable more accurate quantification of the risks.

THE CURRENT STATUS OF GENETIC TESTING FOR BREAST CANCER PREDISPOSITION GENES

How is the test performed?

Genes which have been cloned can be tested by direct gene analysis. This has the advantage of being possible in single individuals, although it is preferable to characterise the cancer-causing mutation in an affected relative prior to testing of those unaffected so as to be able to exclude that they carry that specific mutation. The testing can either be by sequencing or by gene scanning techniques which compare normal and test sequences to detect differences. All the latter have less than 100% sensitivity, but even the gold standard sequencing has to be interpreted with caution. This is primarily the case when looking for point mutations (single base pair changes), since extraneous sequencing bands can be generated, particularly if the gene has glycine- and cystine-rich areas. This can be overcome by demonstrating a point mutation by two alternative methods such as sequencing confirmed by allele-specific oligonucleotide hybridisation.

When the gene is located, but not yet characterised, testing can be performed by linkage analysis using informative markers that flank the region within which the gene lies. This is only possible in large families where many samples are available, although the scope for this type of testing has been extended by the polymerase chain reaction, which enables testing of tissue from stored material from deceased family members. At the time of writing, the BRCA1 gene has not yet been identified so testing for this gene is only possible by linkage. This is only performed in certain high-risk families where linkage to the region containing BRCA1 can be clearly established. In practice, we restrict BRCA1 testing to families with three or more cases of breast cancer (two aged <50 years at diagnosis) plus one or more cases of ovarian cancer since these have the highest probability of carrying the BRCA1 gene. We also require evidence of linkage to BRCA1 using polymorphic flanking markers, such that the probability of being linked to BRCA1 is at least 95%. Once the gene is cloned, testing will be extended to a wider group of at risk individuals who can be tested without having to test multiple family members. At present, many of these people cannot be tested because the linkage analysis is equivocal in their family or there are not enough family members to test to prove linkage at all.

Pretest counselling should be performed and should include an assessment of the risk of the disease with and without the knowledge of the gene carriage status. The breast cancer risk from genetic predisposition decreases as the age of the individual increases because the older they are without having developed the disease, the less likely it is that they carry the gene. This is unlike the cancer risks due to environmental factors.

The screening, prevention and treatment options, and the potential adverse effects of testing should be discussed. These include the possibility of an erroneous result due to limitations of the test (this is very low if two detection methods are used, as described), and the possibility of the gene not causing the family pattern (e.g. <5% risk in the BRCA1 families described above).

Who should be tested?

Currently, genetic testing is available for p53 by direct gene analysis and for BRCA1 by linkage analysis in high-risk families. Guidelines for testing for these genes are being prepared by the U.K. Cancer Family Study Group. This group advises against testing individuals under the age of 18 years for either BRCA1 or p53 mutations. For BRCA1, this causes no difficulty since the cancers caused by the gene occur in adulthood. However, p53 mutations carry a high risk of childhood cancer, for which there are no effective screening methods. Breast cancer due to p53 also occurs in younger individuals, and although this is usually after the age of 18, it often occurs in the early thirties when screening by mammography is less effective. However, this policy has the advantage of avoiding testing in minors who may not be old enough to make decisions about insurance and lifestyle implications.

In principle, when BRCA1 is cloned, one could consider testing individuals at little or no increased breast cancer risk by virtue of family history, for example, individuals with one postmenopausal relative with breast cancer. However, although this may technically be feasible, it is not desirable. The majority of individuals will not be BRCA1 carriers and will be falsely reassured that they do not carry a cancer-causing gene. They may assume that their risk is reduced whereas in fact it is unaltered by the test result.

How should a genetic abnormality be interpreted?

Once a mutation is detected, it has to be shown to cause the disease. Ideally, this is achieved by demonstrating that it segregates with the disease in families, that it is rare in the general population and that it has a functional effect on the resultant protein. This can be difficult to achieve since some genes exhibit rare polymorphisms which have little biological effect. Normal variations, rare in the population (normal polymorphisms), are considered to be normal variants when they occur at a level of 1% or higher. A new mutation, seen for the first time in a cancer family and not seen in a sample of controls, cannot be certain to be disease-causing. The demonstration of an effect on function of the resultant protein is desirable.

Is genetic testing needed in all cases?

A phenotypic marker is already present in some genetic syndromes that carry an increased risk of breast cancer. Such an example is Cowdens syndrome which is a syndrome of multiple hamartomas (multiple benign hair follicle tumours, tongue papillomas and bowel polyps) associated with a small risk of thyroid and gynaecological malignancy and a 50% lifetime risk of female breast cancer [58]. It is caused by an autosomal dominant gene, which has not yet been identified, but gene carriers can be recognised on clinical examination by the presence of their skin and tongue hamartomas.

Skolnick and colleagues [59] suggested that proliferative breast disease as assessed on breast fine needle aspirates segregated with breast cancer in families, but this has not been repeated. If a gene mutation is associated with an abnormal protein product, it may become possible to identify gene carriers by antibody staining of breast cells. However, it has already been shown that, although in tumours a p53 mutation is often associated with positive immunostaining due to stabilisation of the protein, germline p53 mutations are not always accompanied by positive immunostaining of normal cells carrying the mutation [60]. Therefore, in at least one case (p53), genetic analysis is needed for assessing mutations in the germline.

ETHICAL AND SOCIAL IMPLICATIONS

The identification of genes which predispose to cancer and the abiity to test for them in at-risk individuals has posed a number of ethical problems, some of which are the same as those encountered in any genetic testing situation, and some of which are particular to cancer. Unlike in many genetic diseases, there is incomplete penetrance, namely that the carriage of the gene does not mean that the individual has a 100% chance of developing cancer; they are only at increased risk. Conversely, there may be a preventative or early detection measure which can be taken to lessen the effect of carrying a cancer predisposition gene, which is contrary to the situation in, for example, Huntington's disease, where the penetrance is virtually 100% and the disease is incurable. The uptake of genetic testing in Huntington's was low, and both gene carriers and non-carriers suffered morbidity from testing, the latter because they developed "survivor guilt" [61]. Although many preventative measures are unproven, the availability of new chemopreventative measures, even in a trial setting, may result in a higher uptake of genetic testing for cancer predisposition genes.

If oncologists do request BRCA1 testing on every case of breast cancer to make management decisions, this would result in 25 000 test requests per year in the U.K. alone. A more likely scenario is that European oncologists will await the results of genetic testing in defined cancer populations (e.g. two first degree relatives with breast cancer or families with at least one breast and one ovarian cancer) and will then offer BRCA1 testing to groups with a higher chance of carrying the gene as defined by research studies of the gene mutation profile. The situation in the U.S.A. will probably be different because of the different approach to medical tests, and may involve testing of the whole population of breast cancer patients.

It will be very important to define the effects of genetic testing on the ability to obtain life and health insurance. It will be undesirable to reach a situation when a genetic test is not done because it would jeopardise an individual's ability to obtain insurance cover, since, as has been outlined in this article, genetic testing can provide important information for both affected and unaffected family members. As new preventative treatments are found, this will become more pertinent. The ultimate aim is to lower cancer mortality. The other important factor is that some cancer predisposing genes may be common and confer only moderate cancer risks. Testing of large numbers of the population may, therefore, be realised and if chemoprevention reduced a moderate cancer risk to low risk in large numbers of individuals, the overall cancer burden could be dramatically reduced. Discrimination against such genetic testing would be unfortunate and is being urgently addressed. The recent Nuffield report in the U.K. [62] has recommended that insurance companies should adhere to their current policy of not requiring genetic tests as a prerequisite of obtaining insurance. They also recommend that there should be early discussions between the Government and the insurance industry about the future use of genetic data, and that pending this outcome, companies should accept a temporary moratorium on requiring the disclosure of genetic data for policies of moderate size.

In the U.S.A., the insurance implications are wider because of the reliance on private health insurance cover which precludes pre-existing conditions. The U.S.A. health care reforms may introduce cover for these conditions and the situation would then become more like that in the U.K. and other European countries with a national health insurance system.

Research studies of the physical, psychological and financial costs and benefits of genetic testing are needed.

THE ROLE OF THE CANCER FAMILY CLINIC

The identification of genetic components in the aetiology of cancer has led to the establishment of cancer family clinics distinct from general genetics clinics. There are now increasing numbers of these clinics in many European countries, usually run jointly by an oncologist or oncological surgeon and a geneticist. Collaborative groups, such as the French Cooperative Network and the U.K. Cancer Family Study Group, have been established for the study of familial cancer. The French group have proposed a consensus for the management of familial cancer in France (Bignon and Sobol, personal communication).

The role of these clinics is to provide information to families about their risk of cancer because of a positive family history, and where appropriate, to advise and arrange for screening for the various cancers to which the family is predisposed. These clinics also provide information on the availability of genetic tests if indicated, and are a focus for research activities which involve providing samples to identify new predisposition genes, assessing new screening and prevention programmes, and the psychological effects of genetic testing.

CONCLUSIONS

There are several aspects to consider when considering whether a breast cancer patient has familial disease. Is the disease familial at all, i.e. due to a cancer predisposing gene, and if so, which gene is involved? The fact that the disease has genetic heterogeneity is now recognised and advent of the identification of the various breast cancer predisposing genes over the next 10 years will enable direct genetic analysis. This will provide more accurate classification at the genetic level, and will affect the patient's management and the advice given to relatives who are also at risk. It will be very important to have safeguards against discrimination for the patients who wish to have these tests which may determine the management of them and their relatives.

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