Hereditary cancer: guidelines in clinical practice. Breast and ovarian cancer genetics

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Introduction

A variable proportion of cancers have a genetic aetiology but genes rarely act alone in shaping cancer predisposition. Environment and other genes modify risks and much of this interaction is poorly understood at present. Initial genetic risk assessment is often based on knowledge of genetic epidemiology. Different levels of increase in predicted risk may shape clinical decisions about molecular genetic testing, prevention or early detection, and knowledge about available options is important in dealing with cancer patients and their families.

Genetic epidemiology

In common with many cancer sites, twin and population-based studies confirm that a substantial proportion of breast and ovarian cancer cases can be explained by genetic predisposition [1, 2]. Segregation analysis is a computer-based method used to determine the model of inheritance that best fits the observed familial occurrence of a specific disease. For many common cancers where segregation analysis has been applied, low frequency, highly penetrant dominantly inherited gene(s) are predicted [3]. For breast and ovarian cancer, the study of families with multiple cases of breast and sometimes ovarian cancer, led to the discovery of the chromosomal location of the first breast/ovarian cancer gene, BRCA1, on the long arm of chromosome 17 [4]. Four years later the gene sequence was finally published [5]. At the same time, the location of BRCA2 was published and soon after the majority of the gene sequence [6]. These two genes account for the vast majority of families with multiple cases of breast and ovarian cancer but there are still a proportion of multiple breast cancer case families, unlikely to have occurred by chance, where mutations in these two genes seems unlikely to be the underlying explanation.

Current hypotheses favour two likely scenarios that are not mutually exclusive. First, there are likely to be at least one or more highly penetrant rare gene mutations that would explain some of these family clusters. Each faulty gene may, however, be unique to each family and each family is likely to have relatively few individuals available for linkage studies. Putting all the families together in the hope of finding evidence of a single gene location by linkage could not possibly succeed. Studying one very large family, sufficient alone to give a significant result on linkage may, however, succeed where many smaller families would fail. Limited success has been achieved in this way, e.g. the discovery of a specific mutation in the ATM gene [7]. Other genes have been discovered that confer a much lower level of susceptibility to breast cancer than BRCA1 and BRCA2, e.g. CHEK2, but it is still unclear how this type of information can be used appropriately in clinical practice [8].

Secondly, there are likely to be multiple genetic polymorphisms that act to increase risk marginally (relative risk around 1.5–2), and current theories expect that these will have a combined effect on risk. Finding associations between genetic polymorphisms and disease requires very large population samples although using familial cases may reduce the sample size required. Thus far it is unclear how to model the interaction between such mild-effect genes or factor in environmental risk factors to give an individualised risk estimate. It is thought that the majority of individuals with less striking family histories of breast or ovarian cancer may have arisen either through chance or because of this type of multi-factorial predisposition.

Assessment of family history

An accurate assessment of the likelihood that a family history is suggestive of a genetic risk requires knowledge of ages, disease and cause of death for all close relatives. In practice the taking of a family history usually involves documenting details out to third degree relatives (cousins), although in reality it can be difficult to get accurate information about more remote relatives and confirmation of proffered diagnoses is an important part of the assessment of risk. The features of the history that lead to a suspicion of genetic predisposition include unusually young age at onset of cancer, multiple cancers in one individual or multiple blood relatives with cancer at the same or related sites.

In single cases of cancer, even at young ages, if there is a clear family history on both maternal and paternal sides, with female relatives unaffected by breast or ovarian cancer into older age, the chance of an underlying high-risk genetic predisposition is very low. Multiple affected relatives with young average age at diagnosis are most likely to be explained by an underlying genetic predisposition. Breast and ovarian cancer is more likely to be due to BRCA1; male breast cancer cases suggest BRCA2 may be the underlying cause. Occasionally women have presented with fictitious family histories either due to misinformation or misunderstanding of diagnoses in
the family or as a manifestation of Munchausen syndrome [9]. It is, therefore, even more imperative that at least some of the closer affected relatives’ cancer diagnoses are confirmed from medical records in order to prevent inappropriate medical or surgical interventions. Assessment of the family history should be part of routine oncology practice but verification of diagnoses and an extended family history require considerable time and effort and are difficult to achieve in the available time. Specialist cancer genetic services should be able to provide this more detailed service. The likelihood of the family history being due to one of the known high-risk genes determines how appropriate the possible interventions may be. Often women presenting with concerns about their family history want early mammographic surveillance. Relatively few will have a family history compatible with a BRCA1 or BRCA2 mutation. There is a range of available tools that can help in assessing breast cancer risk and the likelihood of a BRCA1 or BRCA2 gene mutation based on the family history. Careful interpretation is necessary and wrong data entry can readily give rise to inflated risk estimates. It is therefore important that individuals using computer software to estimate risk should have a good working knowledge of what they expect the answer to be. Broadly, risk estimation software utilises empiric data or segregation data; different methods give fairly variable results and all are likely to have wide confidence intervals [10, 11].

Surveillance

Breast cancer

The lifetime population risk of breast cancer is around 1 in 10 in the Western world. Mammographic surveillance is offered in many developed countries from 50 years of age. In this age group there is evidence of a mortality benefit but the actual amount of benefit is still the subject of heated debate [12, 13]. The evidence for women under 50 years of age is even more controversial and provision of routine mammography by health care providers varies widely. Surveillance for women under 50 with a family history of breast cancer may be of benefit but so far there is no evidence to show a mortality reduction in this group [14]. In addition, for the high-risk BRCA1 and BRCA2 gene carriers where tumours tend to be high grade and tend not to calcify, mammography may be relatively insensitive leading to current trials examining the place of magnetic resonance imaging for early diagnosis of breast cancer in this high-risk group [15].

Ovarian cancer

The lifetime population risk for ovarian cancer is ~ 1 in 75. A BRCA1 gene carrier has a ~ 1 in 2 risk of developing the disease and for a BRCA2 gene carrier the lifetime risk is ~ 1 in 5. Studies have looked at various modalities for early detection of ovarian cancer including pelvic ultrasound scanning and serum screening for tumour markers. A major problem for early detection of ovarian cancer is that there is no clearly recognised pre-malignant stage to target. None of the available methods have been shown to detect disease at an earlier stage overall compared to ovarian cancer presenting with symptoms, although adequately powered studies are lacking at present. Abnormal ultrasound and serum CA125 measurement often lead to unnecessary interventions, often surgical laparoscopy or salpingo-oophorectomy. A recent study of proteomic serum analysis has focused current interest on this as a potentially useful approach [16].

Prevention

Medical prevention

Tamoxifen has been found to reduce the risk of oestrogen-receptor-positive breast cancer in a number of prevention studies recruiting women at increased risk for breast cancer [17]. Unfortunately, as yet there is no evidence about the long-term risk–benefit balance and there are undoubtedly unwanted effects from this drug, including an increased risk of venous thrombo-embolism and endometrial carcinoma. Tamoxifen as adjuvant therapy in BRCA1 gene carriers reduced the risk of contralateral breast cancer [18], but is often not used as adjuvant therapy in BRCA1-related breast cancer as 80% of these are oestrogen-receptor-negative. For BRCA1 or BRCA2 where the age at onset is frequently pre-menopausal, earlier intervention may be necessary. Trials of intervention using gonadotropin-releasing hormone (GnRH) agonists with ‘add back’ remain of interest, but trials of such interventions seem to be an unattractive option to younger women [19].

Surgical prevention

Risk-reducing surgery for the breasts and/or ovaries and fallopian tubes may be the preferred option for some women. Uptake rates vary widely, influenced by a number of factors including age, parenthood, health beliefs and cultural attitudes [20]. Risks for future cancer can be reduced greatly, although probably not eliminated completely, and thorough assessment before surgery with imaging and after surgery with careful pathological examination for occult malignancy is essential [21]. Removal of the ovaries alone is insufficient: the fallopian tubes must be removed since the risk of fallopian tube cancer is greatly increased above the population risk in BRCA1 and BRCA2 gene carriers and abnormalities of fallopian tube epithelium are common in prophylactically removed fallopian tubes [22].

BRCA1 and BRCA2 genes

The BRCA1 and BRCA2 genes are both very large and, apart from mutations arising on a background of common ancestry, mutations tend to be unique to each family. The likelihood of developing breast or ovarian cancer may vary according to type and position of mutation and may vary in individuals according to other genes that modify the effect of the high-risk genotype or environmental modifiers. These modifying
effects are difficult to identify and the magnitude of their effect in each individual is difficult to measure. Studies have suggested, for example, that in a BRCA1 gene carrier variation in the length of a repetitive stretch of DNA in the androgen receptor gene may modify the risk for breast cancer and that use of the oral contraceptive pill may increase breast cancer risk [23, 24].

Other breast cancer genes

Other high-risk known genes that predispose to breast cancer tend to be rare and associated with specific clinical features on which a diagnosis may be made. These include Li–Fraumeni syndrome (also associated with soft tissue sarcoma, adrenocortical carcinoma, glioblastoma and lung cancer, gene TP53), Peutz–Jegher syndrome (diagnosed from the association of gastrointestinal tract hamartomas and skin and mucosal pigmentation, gene LKB1/STK11) and Cowden disease (usually associated with macrocephaly, trichilemmomas and other features, gene PTEN) [25]. There are other genes associated with breast cancer risk including the gene for Ataxia Telangiectasia and CHEK2, which are thought to confer an increase in risk although it is difficult to quantify this risk accurately. Thus, the availability of a genetic test does not necessarily mean that it will be useful or cost effective to perform this test in all circumstances.

Molecular tests

In general, testing for lower-penetrance genes is not currently clinically meaningful and therefore not available as a diagnostic service. Testing for mutations in BRCA1 and BRCA2 has, however, become routine practice in many diagnostic clinical genetics services. Clinical selection criteria are utilised to select cases with the highest likelihood of detecting a mutation in order to limit the cost of testing in a clinical setting and to direct resources at families likely to benefit most from this health intervention. Selection of families with a single case of breast or ovarian cancer with clearly no history of the disease on either side of the family, even if very young, would be expected to yield very few mutation carriers [26–28]. Ideally a sample from an individual from a family affected with cancer at a young age will give the best chance of identifying the specific causative mutation. Mutation analysis is not straightforward. Both the BRCA1 and BRCA2 genes are unusually large, a mutation might be anywhere in the coding or even non-coding regions of the gene, no single method for genetic testing reveals all possible mutations and even the more sensitive techniques probably have <90% sensitivity for all possible mutations. Many mutation analysis methods rely on comparison of the normal (wild-type) sequence on one allele compared to the other (mutant) allele in an individual to highlight a few sections of the gene worth analysing further by DNA sequencing. Direct sequencing of all coding exons of both genes might be expected to pick up all mutations but the recent development of new techniques reveals that possibly 5–10% of mutations are large deletions involving one or more whole exons: such large deletions would not be detected by sequencing since only the wild-type allele would be present and so only the wild-type sequence would be detectable.

Any approach to mutation analysis may result in a number of possible outcomes. A clearly pathogenic, usually protein-truncating mutation, might be found. This is the most useful outcome for the wider family as it gives them the option for a definitive, meaningful predictive genetic test. Sometimes a sequence variant is found in the individual that is not known to be a common variant in the population but where the effect of the mutation on the predicted protein is too subtle to know whether it might affect the function of that protein. Further functional testing may be possible but is largely research-based. Finally, the testing may show no genetic variation from the expected wild-type sequence. In this case it may be that the cancer occurred by chance and not because of a genetic predisposition in that individual (sampling and testing a second young individual from the family would be appropriate if the family history is extensive as sporadic cases do occur within high-risk families). Alternatively, the family history may be due to genes, other than BRCA1 or BRCA2, that have not been tested for or may not yet have been discovered. Other high-risk breast cancer genes, including, potentially, genes with dominant, recessive and polygenic modes of inheritance, probably do remain to be discovered.

At present since no genetic testing process can be claimed to be fully comprehensive and 100% sensitive, genetic testing in this context cannot rule out the possibility of a genetic risk in a family. In some families there is no living, affected relative from whom a DNA sample can be obtained. DNA in pathology blocks tends to be poor quality and difficult to work with, and on the whole this material is unsuitable for looking for subtle errors across a large amount of sequence. It is, of course, possible to test a blood sample from an unaffected at-risk family member, but if sequencing of both BRCA1 and BRCA2 shows no abnormality, the interpretation of this result may be difficult. As outlined above, even for an affected cancer patient interpretation can be far from clear, but with the added uncertainty that if a genetic mutation was present in the family the unaffected individual only had at most a 50% chance of having inherited the gene fault. The only confident statement that can be made from a negative outcome for mutation testing is that they are fairly unlikely to be at very high risk for ovarian cancer.

Knowledge of ethnicity and groups with known common ancestral gene mutations can provide helpful clues to a possible underlying mutation and in some cases can make genetic testing in an unaffected individual more meaningful than in an ethnically diverse population. For example, in Eastern Europe the 5382insC mutation in BRCA1 is very frequently reported. This mutation is also one of three frequently detected Ashkenazi Jewish mutations, the others being 185delAG in BRCA1 and 6174delT in BRCA2. Such mutations arising on a common background haplotype are due to a mutation many generations previously in a ‘founder’ ancestor and tend to
become common where there has been a severe reduction in population size and then rapid expansion, or where genetic isolation arises due to either geographic or cultural restraints on marriage. Founder mutations have been reported for many countries and ethnic groups worldwide.

**BRCA1 and BRCA2 protein function and cancer treatment**

Since the discovery of the **BRCA1** and **BRCA2** genes there has been an enormous literature investigating gene function and their roles in site-specific carcinogenesis. The whole picture is far from clear but several interesting observations have been made, although most studies in humans are hampered by a retrospective design, limited genetic-testing options and incomplete or incomparable data.

Several groups have reported that the pathology of **BRCA1**, and perhaps to a lesser extent **BRCA2**, differs overall from sporadic breast cancer. As a group, **BRCA1** gene carriers will develop breast cancer that is more frequently oestrogen-receptor and progesterone-receptor negative, HER-2 negative and grade 2 or 3 compared to the average [29]. In addition, 13% of **BRCA1**-associated breast cancers are described as medullary or atypical medullary due to a prominent pushing tumour margin and tumour-infiltrating lymphocytes. This compares with 1–3% in routine practice. More recently extensive immunohistochemical profiling suggests that pathological features consistent with a basal epithelial type of breast cancer are typical of **BRCA1** [30]. No feature has been described that is pathognomonic for **BRCA1**-related tumours. For **BRCA2**, the features are less distinctive. Although higher grade tumours are typical these are more likely to be oestrogen-receptor positive (up to 80% of tumours associated with inherited **BRCA2** mutations) [29].

Retrospective studies of **BRCA1**, **BRCA2** and familial breast cancers compared to population-based samples are, on the whole, methodologically flawed and give conflicting results [31–33]. More recent studies suggest (as might be predicted from the pathology) that **BRCA1**-associated tumours may have a worse prognosis than average and **BRCA2** may be a little better than average [34]. No data comparing prospectively the outcome for gene carriers with controls matched for all other major prognostic factors have been published. Some studies have looked at the risk for contralateral breast cancer in gene carriers or in familial cases, and there is clearly an increased risk of up to 40% for contralateral breast cancer in the first 10 years after initial diagnosis [31, 35]. The picture for ipsilateral breast tumours after breast-conserving treatment is still unclear: several studies suggest no increase in risk with median follow-up ~8–10 years [31, 36–38]. Longer-term studies are needed and should focus on whether radiotherapy increases the risk for other cancers within the therapeutic radiation field (no evidence for this at present), the influence of second cancers on long-term survival and the effect of adjuvant therapies for the initial primary on second cancer incidence. Limited data indicate that, despite the predominance of oestrogen-receptor negative tumours in **BRCA1** carriers, adjuvant tamoxifen reduces the risk of contralateral breast cancer by as much as 50% [39, 40]. Physicians rarely prescribe tamoxifen as adjuvant therapy for **BRCA1** gene carriers since these tumours are often oestrogen-receptor negative [40].

The **BRCA1** and **BRCA2** gene products are both involved in cellular mechanisms aimed at recognition and repair of damaged DNA, in particular double-stranded DNA breaks. In vitro experiments suggest that **BRCA1** and **BRCA2** null cells are unusually sensitive to the DNA cross-linking agents cisplatin and mitomycin C but resistant to taxanes [41]. Limited retrospective data, mainly in women of Jewish descent, indicate that adjuvant chemotherapy has a greater beneficial effect in **BRCA1** gene carriers than non-carrier breast cancer patients [42]. Gene expression micro-array experiments suggest that **BRCA1** and **BRCA2** null tumours may have a specific gene expression signature [43, 44]. If new technologies make it possible to identify **BRCA1** or **BRCA2** null breast cancers at the point of diagnosis, whether due to an underlying inherited mutation or not, it might provide a practical way to approach therapeutic trials recruiting women with specific tumour gene expression profiles at the point of diagnosis without needing to know or even test for an underlying inherited mutation.

**Clinical genetics services in Europe**

It is only a decade since the **BRCA1** gene was discovered. Although clinical genetics has existed as a medical discipline for much longer than this, specific cancer genetics services have arisen largely to meet the demands for genetic testing and advice to patients, families and clinicians concerned about future cancer risks. In some countries, such as the UK, cancer genetics services for high-risk predisposition are offered by regionally based specialised clinical genetics services. In other countries, such as Germany and Switzerland, the cancer genetics services are provided by clinicians and counsellors within the oncology service with a special interest. There is now a wealth of literature aimed at the lay public available from cancer charities and via the worldwide web. Referrals for genetic advice are rapidly increasing and the majority of those expressing concern at primary care level are likely to fall into the category of sporadic cases or familial clustering of low-penetration genes. It is inappropriate to try and deal with the bulk of these within expensive specialist services where genetic testing is unlikely to be of any benefit. If risk is sufficiently increased, surveillance may be offered to at-risk family members. Where family history reveals a likely high-risk genetic predisposition then the input from a specialist service with the experience and molecular back-up to provide appropriate and accurate assessment and information may be of benefit regardless of where such services are based.

**Future perspectives**

In another decade or so, genetic risk profiling may be able to partition populations into those at higher levels of risk for
a common disease like breast cancer and those with a very low risk. Factoring in environmental risk factors to individualised risk prediction adds another layer of complexity but many of these environmental risk factors are already well recognised although not really utilised in making decisions about risk management on the whole. It is likely that most, if not all, of the high-risk breast cancer predisposition genes will have been mapped and their sequence will be known. It is to be hoped that non-toxic, practical and effective medical options for reducing cancer risk will become available and judicious use of such agents may make it possible to delay or avoid surgical risk reduction options. A better knowledge of the precise role of the BRCA1 and BRCA2 genes in carcinogenesis may help to understand the differential effect of cancer therapies. In more general terms, genetic variation may explain a substantial proportion of unpredicted therapeutic or toxic drug effects and more knowledge of pharmacogenetics may help to individualise treatment, either through profiling of the host genome or tumour genotype or both.

Summary

The discovery of two major breast and ovarian cancer-predisposing genes has led to the introduction of genetic testing for risk prediction into routine clinical practice. The measures available to address an increase in breast and ovarian cancer risk are still relatively limited and the overall benefits in terms of mortality reduction in particular are still controversial. In the clinic, efforts to clarify the many areas of uncertainty will be facilitated by well-designed, comprehensive prospective studies, and close collaboration between clinicians and laboratory scientists is key to rapid and meaningful progress.

References