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Screening for Hereditary Cancer and Genetic Testing, Epitomised by Breast Cancer

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The new genetics is having an impact on many areas of healthcare. Diversity in the genetic code accounts for differences in phenotypes between populations and it is becoming apparent that genetic differences may have a role in predisposition to and behaviour of disease. Genetic models suggest that there are two types of genetic predisposition to disease: the so-called high and low penetrance genes. At present, most of the impact on medicine has been from highly penetrant genes, and genetic testing for disease predisposition, particularly for diseases of late onset (e.g. certain cancers) is in its infancy. As a general statement, approximately 5–10% of common cancers are due to such highly penetrant genes. The category of genes that will become of increasing interest is that of the low penetrance genes. Often these are normal variations in genes that result in a slightly increased risk of disease. These are analogous to high blood pressure carrying an increased risk of cardiovascular disease. Once rapid genetic analysis is available for these types of genes, such analysis would be analogous to taking someone's blood pressure in a general practitioner's (GP's) surgery to identify individuals at increased risk of cardiovascular disease. This will produce a revolutionary change in the way we practise medicine. Genetic analysis will become faster and may therefore be more commonplace. It is possible to envisage an era when genetic analysis will become a routine part of primary care to identify changes in low penetrance genes that will confer a 'risk profile' for patients. This will then enable their primary care physicians to advise about primary prevention and even prescribe certain preventive drugs to decrease the risk of certain diseases occurring. This proactive rather than reactive style of practising medicine is potentially exciting, however it carries with it ethical, legal and social implications for how we deal with this new knowledge. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

In the U.K., there are 25 000 cases of breast cancer and just over 5000 cases of ovarian cancer per year [1]. Cancer has been known to 'run in families' for many centuries. It was described in Roman times and Broca, in 1866, described familial breast cancer in his wife's family [2]. In one sense, all cancer is genetic at the cellular level since genetic changes occur when a normal cell is transformed into a cancer cell; however, these changes are usually somatic (they occur only in the cancer cells and are not present in normal cells). A proportion of the common cancers (usually 5–10% [3]) occur in individuals with a germline genetic alteration which confers an increased risk of cancer development. This is termed 'genetic predisposition to cancer' and the genetic alterations occur in every cell in the body (with the exception of some of the gametes), as they are in the germline. Such alterations can also be passed on to offspring since, on average, half the gametes will contain the genetic alteration.

The clustering of more than one case of cancer within a family ('familial cancer') can occur for several reasons: (i) clustering may occur due to chance; this is particularly seen with the common cancers. The higher the prevalence of a disease, the more likely it is that clustering will occur in families due to chance alone; (ii) there may be a cancer predisposition gene in the family accounting for familial clustering; (iii) individuals in the same family may share the same carcinogenic environmental exposures.

WHEN IS FAMILIAL CANCER MORE LIKELY TO BE DUE TO A CANCER PREDISPOSITION GENE IN THE FAMILY?

The markers of the presence of a cancer predisposition gene in a familial cluster are as follows:

- The clustering together of cancers at sites that are normally rare in the general population (e.g. the association of medullary thyroid cancer with phaeochromocytoma in the multiple endocrine neoplasia type 2 syndrome).
- Young age of onset relative to the general population.
- The occurrence of cancer of the same type on more than one occasion in the same individual (e.g. bilateral breast cancer; multiple colonic cancer).
- The presence of multiple cases of cancer in several individuals on the same side of a family.

HIGHLY PENETRANT GENES

Approximately 5–10% of breast and ovarian cancers occur as a result of highly penetrant germline mutations in cancer-predisposition genes [3–5]. One of these genes, *BRCA1*, predisposing to breast and/or ovarian cancer, was mapped to the long arm of chromosome 17 in 1990 [6, 7] and cloned in 1994 [8]. Collaborative studies by the Breast Cancer Linkage Consortium (BCLC) have shown that *BRCA1* mutations are responsible for approximately 50% of families with clear dominant predisposition to breast cancer and over 80% of families segregating both breast and ovarian cancer [9, 10]. A majority (32% of the total [10]) 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 [11]. It is known that there is at least one more highly penetrant breast cancer gene that may account for just under half of high-risk families with numerous cases of early onset breast cancer but may account for up to 80% of families with at least three cases of breast cancer under 50 years (D. Easton, CRC Genetic Epidemiology Unit, Strangeways Lab., Cambridge, U.K.). The remaining gene(s) has been named *BRCA3* and has not yet been found. It is very likely that there is more than one remaining gene to account for high-risk families with at least three cases of breast cancer under 60 years of age. The current situation is therefore very imperfect since only some familial breast cancer clusters due to genetic alterations will be able to have these identified (Table 1).

Currently, highly penetrant genes are located by linkage analyses. These are the co-segregation of genetic markers (variable runs of bases within the genome) with the disease. If the variation in the markers is inherited in the same way as the disease, the disease gene is said to be linked or close to the marker, the location of which is known [12].

BRCA1 has 24 exons of which 22 code for a protein of 1863 amino acids [8]. Over 100 distinct mutations in *BRCA1* have been described to date [13–15]. These mutations are widely scattered across the gene (Figure 1). A very similar mutation pattern occurs in *BRCA2* (Figure 2) which is approximately twice as large. These mutations 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 is consistent with the hypothesis that *BRCA1* acts as a tumour suppressor gene [16]. 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 large majority of mutations in *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. Thus, the absence of a *BRCA1* mutation in the

Table 1. *Threshold for probability of* BRCA1/2 *mutation*

Chance that a mutation is present*	Clinical criteria
< 10%	All single cases of breast or ovarian cancer
10%	Single breast cancer cases < 35 years of age
> 10%–≤30%	2 breast cancer cases < 50 years of age 1 breast cancer < 40 years of age in an Ashkenazi
≤50%	3 breast cancer cases < 50 years of age 4–5 breast cancer cases, no ovarian cancer 1 breast and ovarian cancer
> 50%	> 1 breast and ovarian cancer ≥4 cases of female/male breast cancer > 6 female breast cancer

Sources: [10, 20, 21] and Frank TS, Manley S, Thomas A, and colleagues, *BRCA1* and *BRCA2* sequence analysis of 335 high-risk women. Presented at the 47th Annual Meeting of the ASHG, 1997.
*The chance of detecting a mutation is lower because at least 15% of mutations are regulatory, i.e. are not in the coding region of the gene which is the area tested; and the genetic screening methods are approximately 80% sensitive.

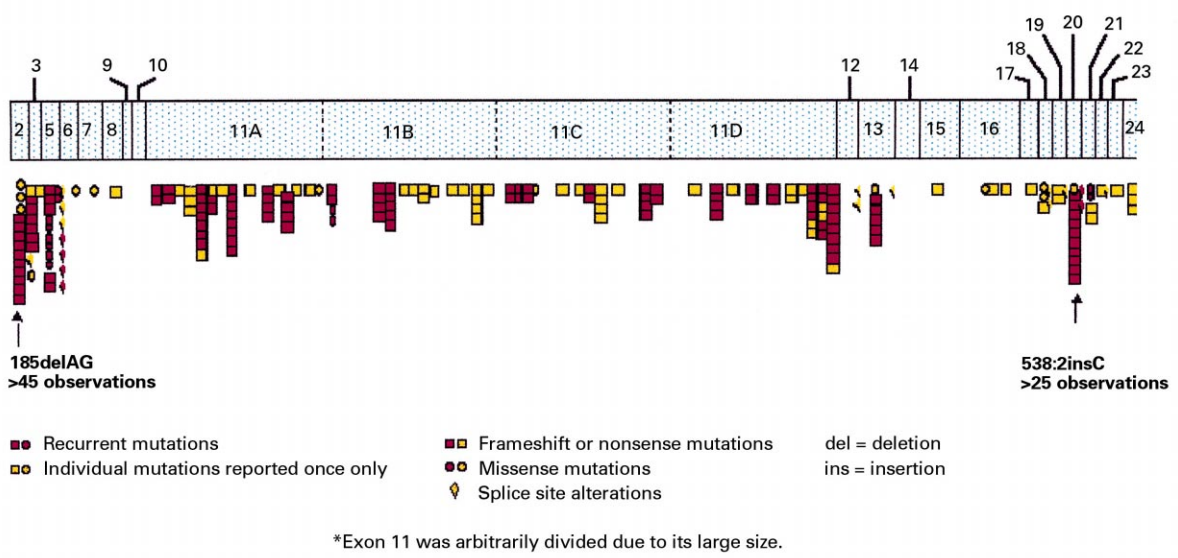

Figure 1. ***BRCA1*** **condensed mutation database from BIC database [15].**

coding region/splice sites, even after rigorous sequencing, cannot rule out the possibility that the high risk of breast cancer in a family is due to *BRCA1*.

The causal nature of frameshift and nonsense mutations is clear from the fact that such mutations are present in a high proportion of *BRCA1* linked families, but in a very low proportion of the general population, and from the consistency with the tumour suppressor gene hypothesis. The missense mutations are more problematic, firstly, because the biological effect of such mutations is less certain, and secondly, because a number of sequence variants in *BRCA1* have been observed in unselected, unaffected individuals. The effect of these variants (some of which are quite rare) on disease risk is uncertain. Thus, a missense mutation in a *BRCA1* linked family may be a rare polymorphism. These rare variants form 34% of *BRCA1* mutations and 38% of *BRCA2* mutations [17].

In the absence of any biological assay, the effect of such a sequence variation can only be assessed reliably by determining its frequency in a series of families or cases, and a large series of appropriate, ethnically matched controls. At present, the only missense mutations where the evidence for causality is beyond reasonable doubt are those affecting the cys residues at codons 61 and 64. These mutations have been observed in several *BRCA1* linked families, and affect the ring finger motif. Splice site mutations can also be problematic if they lead to splicing out of an exon rather than to a stop codon, because the splicing out of an entire exon could be a normal alternative transcript. As in the case of missense mutations, evidence that such splice-site alterations are disease causing is equivocal unless they have been seen in several *BRCA1* linked families and are absent in a large series of controls.

The carrier frequency of *BRCA1* mutations in the general U.K. population has been estimated indirectly from epidemiological studies at approximately 1 in 800, with the range of plausible estimates being 1 in 500 to 1 in 2500 [18]. The corresponding estimated frequencies amongst breast and ovarian cancer cases at different ages are shown in Table 2. Studies of early onset breast cancer cases in Boston and Seattle have found *BRCA1* carrier rates which are in good agreement with these estimates [19, 20]. Much higher frequencies are known to apply to Ashkenazi Jews, owing to a founder effect involving mainly a particular mutation (185delAG [21]). This mutation has an estimated frequency of approximately 1 in 100 in Ashkenazi Jews, and approxi-

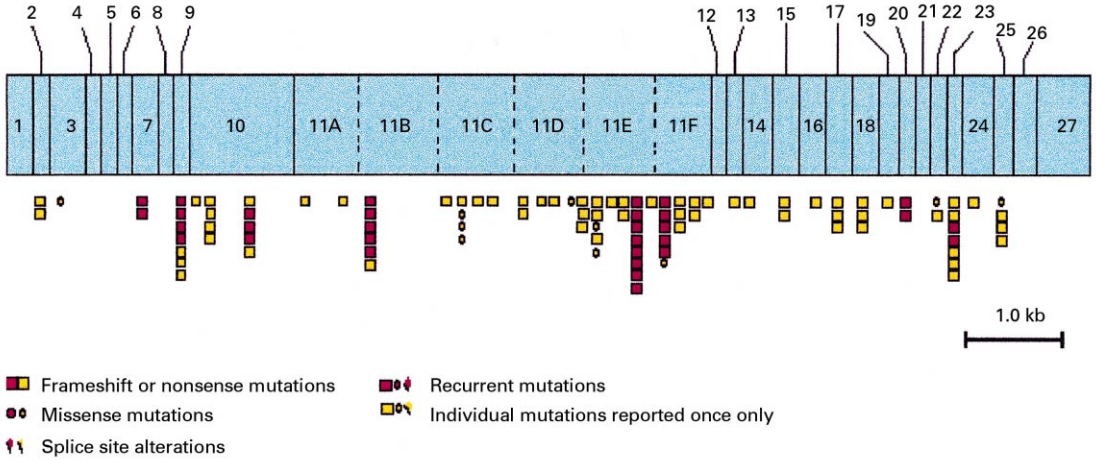

Figure 2. ***BRCA2*** **condensed mutation database from BIC database [15].**

Table 2. Proportion of single cases due to BRCA1 *by age estimated from the gene frequency*

Age (years)	Breast %	Ovary %
20–29	7.5	5.9
30–39	5.1	5.6
40–49	2.2	4.6
50–59	1.4	2.6
60–69	0.8	1.8
20–69	1.7	2.8

Source: Ref. [18].

mately 1 in 5 in Jewish women diagnosed with breast cancer under 40 years of age [21, 22]. This estimated frequency rises to at least 2 out of 3 Ashkenazi Jewish families with breast and ovarian cancer [23]. There is another mutation in *BRCA1* which is more commonly seen in the Ashkenazim (5382insC) and another in *BRCA2* (6174delT) which is present in 1 in 75 of this population [24–26].

Studies by the BCLC have provided estimates of the cancer risks in carriers of mutations in *BRCA1* and these figures can be used in counselling [9, 10, 27, 28]. Based on the incidence of disease in linked families, Easton and colleagues estimated the risk of breast cancer to be 51% by the age of 50 years and 85% by the age of 70 years [28]. These high risks have been confirmed by studying the risk of contralateral breast cancer in carriers who have already developed one cancer [27], and by a study of a large Utah kindred [29]. The risk of ovarian cancer has been estimated to be 63% by the age of 70 years, 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 [28]. However, there is some evidence that the ovarian cancer risk is mutation dependent [28]; Gayther and colleagues have found that mutations towards the 5′ end (the beginning) of *BRCA1* confer a higher risk of ovarian cancer than those towards the 3′ end [30]. In our opinion, the best risk estimates for counselling purposes at the present time are those derived from the BCLC studies, i.e. a risk of ovarian of 63%, ignoring the possibility of allelic heterogeneity (the fact that different mutations may have different risks). This situation may change, however, if the evidence of allelic heterogeneity becomes stronger. Furthermore, there is increasing evidence that the phenotype of the family may determine the true risk profiles for unaffected *BRCA1* carriers. For example, those clusters with multiple cases of ovarian cancer may have a higher ovarian risk than those with breast cancer alone. The risk of ovarian cancer in *BRCA2* is also raised (27% by the age of 80 years) but not as high as for *BRCA1* [10]. The population risk of ovarian cancer is $< 1\%$.

There is also some evidence of increased risks of colon and prostate cancer in *BRCA1* gene carriers. At least two *BRCA1* families are known to contain male breast cancer cases but the risk appears to be substantially lower than for *BRCA2*. *BRCA2* confers a 5% lifetime risk of male breast cancer. The risk of colon cancer in *BRCA1* is estimated to be increased 4-fold, and the risk of prostate cancer is estimated to be increased 3-fold, corresponding to an absolute risk for each cancer of approximately 6% by the age of 70 years [27]; in *BRCA2* the prostate cancer risk is 6–14% by the age of 70 years [31, 32].

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. 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 in 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 one 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 mutation 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 approximately 16% [33]). In research families, the uptake of *BRCA1* testing is overall, approximately 44% and is higher in women than in men [34–36]. One year after the result no adverse psychological features were observed [36].

Some models suggest that up to 80–90% of common cancers may be partly due to a genetic component due to the contribution from low penetrance genes which confer a slightly increased risk [3]. In people ascertained from the general population the breast cancer risk estimate due to mutations in *BRCA1* is much lower (37–50%) [37, 38]. 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 years without cancer, whilst others present with breast cancer in their 20s. Many environmental factors such as diet, exercise, hormonal and carcinogen exposure and unknown external factors could be responsible for these differences. The BCLC is currently studying these.

MANAGEMENT OF WOMEN AT INCREASED BREAST CANCER RISK DUE TO A GENETIC PREDISPOSITION

There are currently several approaches to the management of increased breast cancer risk due to a genetic predisposition. These are:

- Early detection through screening programmes
- Change in lifestyle
- Chemoprevention
- Prophylactic surgery

Early detection through screening

At present, the only clinical manoeuvres that are offered in cancer genetics/breast risk clinics are the teaching of breast awareness and earlier mammography. Population mammographic screening is offered three-yearly from the age of 50 years as it has been shown to reduce mortality by at least 20% (summarised in [39]). Earlier mammographic screening, often offered annually from the age of 35 until 50 years has been suggested by the British Association of Surgical Oncol-

ogists and the Cancer Family Study Group [40]. It has been shown that at least as many cancers are detected per thousand women screened, in women under the age of 50 years offered mammographic screening because of their family history, as are found in the national screening programme in women over 50 years of age [41].

New methods of breast screening in high-risk women are being investigated, in particular the use of magnetic resonance imaging [42] and analysis of nipple fluid contents [43].

Lifestyle issues

The interaction of hormones with the *BRCA1/2* genes has been studied in cell lines. The *BRCA1* mRNA and protein *in vitro* is increased upon stimulation with sex steroids [44]. *In vitro*, the expression of *BRCA1* mRNA in murine mammary gland is 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 [45]. Thus, hormones might enhance malignant transformation in the presence of a mutated gene.

Few studies have tried to investigate the risk conferred by various hormonal factors in human *BRCA1/2* mutation carriers due to their relative rarity. In a historical cohort [46] 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 the year of birth after 1930. It could be that different demographic factors, as yet unknown, elevate the risk for breast cancer in gene carriers.

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. 42 were non-carriers. Furthermore, 67 of the control group underwent bilateral oophorectomy at an average age of 45 years old [47].

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 in this study the use of oral contraceptives might increase the risk for breast cancer in *BRCA1/2* carriers more than in non-carriers [48].

A provoking article, recently published, reported the influence of smoking on the risk of breast cancer in *BRCA1/2* carriers [49]. 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. It therefore has an anti-oestrogenic effect that might have a protective role in *BRCA1/2* carriers. Obviously, the other carcinogenic effects of smoking preclude its use as a preventive strategy, but the underlying mechanism provides supportive evidence that anti-oestrogenic chemopreventive manoeuvres could be effective in *BRCA1/2* carriers. Recent supportive evidence has been provided by Rebbeck and coworkers who showed that prophylactic oophorectomy reduced breast cancer risk by at least a half [50].

Chemoprevention

Three recent chemoprevention trials have reported conflicting results [51–53]. 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 preventive agent would have been expected to act, and Powles' and Veronesi's studies did not show this. The 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.

Prophylactic surgery

The role of prophylactic surgery in high risk breast cancer gene carriers is unproven; however, there are early data that are suggestive that it will confer some reduction in risk. The largest study of prophylactic mastectomy is that from Hartmann and colleagues who suggested a risk reduction of breast cancer of at least 90% after prophylactic mastectomy [54]. The problem with this study is that very few women had had a genetic test and therefore their exact genetic status was unknown.

Prophylactic oophorectomy in high-risk women is thought to reduce the risk of ovarian cancer considerably from a range of 16% (the lowest risk figures from the Jewish mutations in *BRCA1/2* [55]) to 60% (the highest figures from *BRCA1* lifetime ovarian risks [10, 28]) to approximately 2–3%. The risks are not reduced to exactly those of the general population (just less than 1%) because of the risk of peritoneal adenocarcinomatosis [56, 57].

LOW PENETRANCE GENES

Low penetrance genes are genes which, in their altered form, confer a slightly increased risk of disease. Usually, these are genes with normal alternative forms of their sequences (polymorphisms) which code for different functional activities of the resultant protein. Since the differences in the sequences are a normal variant or polymorphism, this would not traditionally be classified as being cancer-causing. However, the polymorphism codes for a different function of the resultant protein which can result in increased risk of cancer development. An example is the variation in the *Cyp17* oestrogen metabolism gene which confers an increased risk of breast cancer [58]. Such alterations are normally discovered by association studies which investigate the proportion of cases of a particular disease harbouring the polymorphism or variation in normal genetic sequences compared with controls. The problem is one of statistical power. If subtly increased risks are being conferred by the genetic alteration then large numbers of cases and controls are needed to determine the true effect of the polymorphism. The literature is currently mushrooming with small studies suggesting low

penetrance gene effects which are then subsequently discounted by further studies. Very large-scale genetic analyses will be needed and the advent of robotics will revolutionise this area of genetic research.

LOOKING FORWARD TO THE NEXT MILLENNIUM

The completion of the Human Genome Project may be accomplished as early as the first year of the next millennium (Francis Collins, Late-breaking News Session, American Society of Human Genetics, U.S.A., 1999) and the code of all the expressed human genes will subsequently become known. This will include both the high- and moderate- to low-risk genes which increase breast cancer risk. The limitations will be in the following areas:

- The effects of gene–environment interactions (genetic epidemiology).
- The technology to discover the altered genetic sequences and their subsequent functional effects.
- Ethical, legal and psychosocial issues.

Genetic epidemiology

The genotype is the genetic make-up of an individual or cell (the DNA code) and the phenotype is the physical or biochemical effect of the genotype (e.g. the occurrence of a certain type of cancer in an individual with a genetic alteration that predisposes to cancer). The presence of an alteration in a cancer predisposing gene increases the risk of cancer development but does not always mean that the disease will occur. The problem with our current level of knowledge is that cancer-causing alterations can be detected in some cancer predisposition genes but it is unknown whether cancer will definitely occur. Furthermore, even within families where a cancer predisposing gene is known to be present, cases can still occur due to chance, not due to the presence of the altered gene (so-called phenocopies). This will alter as the Human Genome Project progresses and the complete genetic sequence related to breast cancer predisposition can be determined for one particular individual. The identification of phenocopies will then be possible. Research will concentrate on gene–environment and gene–gene interactions that modify the risk conferred solely by one alteration in the genetic code. This will be a major development in the next decade.

Technology

Improvements in technology can be divided into two areas. The first is the development of more rapid methods of analysis of human genetic sequence. The greatest advances in this area have been made in the area of 'chip technology' [59]. Short runs of normal genetic sequences are aligned on a chip or glass slide and the technology is analogous to the chip technology used in computers. Hybridisation with the test sequence which is fluorescently labelled, highlights areas of mismatch where the test sequence code does not have the same read-out and this can be read by fluorescent detectors. The limitations of this technology are currently its sensitivity and lack of specificity. Improvements in this, together with large-scale robotics may enable very fast through-put genetic analysis of individuals' DNA. DNA tests which currently take years could take weeks or even days.

The second area is that of analysis of the functional effect of a change in the genetic code. Cancer-causing alterations are often alterations in the genetic sequence that result in a truncated protein (usually insertions/deletions of genetic code or nonsense mutations which convert an amino acid code to a stop codon). Missense mutations that alter one amino acid to another but do not result in a change in the length of the resultant protein are more problematic because they could be normal variants of the code (polymorphisms) or result in a disease-causing alteration. Traditionally, a polymorphism is classified as a variant with a frequency of more than 1% in ethnically matched individuals. However, 34–38% of the variants listed in the website for the mutations in *BRCA1* and *BRCA2* are missense mutations, which have not been found in 1% of the normal population [15]. These are, therefore, called variants of unknown significance. When an adequate functional assay is developed, the variants that cause a change in function will be able to be identified.

Data from recent studies indicate that *BRCA1* and *BRCA2* have a primary role in DNA damage response by processing signals that arise after damage [60]. Current *BRCA1/2* functional assays involve transfection of different vector constructs in yeast/human cell lines and can only look at part of the gene [61–63]. Although this is an advance towards a functional assay for *BRCA1/2* function, only part of the gene can be analysed in each assay, and it will only determine the function of coding mutations. At least 15% of mutations are regulatory. These 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. Thus, the absence of a *BRCA1* mutation in the coding region/splice sites, even after rigorous sequencing, cannot rule out the possibility that a family is due to *BRCA1*. When a superior functional assay is developed, there will be no requirement to analyse genetic sequences and the regulatory mutations will be detected.

Ethical, legal and psychosocial issues

The rapid advances in knowledge about the genetic code that may predispose to disease and the ability to analyse the code faster using improvements in technology could theoretically result in doctors being in the position of being able to offer rapid genetic analyses to large numbers of the population and provide a 'risk profile'. Theoretically, the entire human genome could be arrayed on a single chip (the 'biochip') and limitations on its use will be determined by cost, the usefulness of the information and 'the desire to know'.

Genetic testing for breast cancer predisposition has shown that there is an increased interest in testing by women of higher social class and this is proportionate to the level of anxiety [64–66]. Risk perception is a complex issue and is not directly related to the absolute level of risk [67]. If there were a demand for genetic profiling to determine risk profiles so that individuals could take preventative measures to avoid disease, the next issue is at what age such a profile should be offered. Currently, testing of children less than 18 years is only offered if it would alter medical management, such as in the familial polyposis or multiple endocrine neoplasia type 2 syndromes [68, 69]. Prenatal testing has been offered for some familial cancer syndromes [70]; however, there is a lot of controversy in this area because the tests are for late-onset disorders and with therapeutic improvements, these disorders may be curable by the time the child is an adult. Germline manipulation of genetic alterations is illegal.

Summary Box 1
Genetic Predisposition to Breast Cancer: the Present

- Only 5–10% of breast cancers occur in individuals with a high risk breast cancer predisposition gene.
- Of these 5–10%, approximately half are due to *BRCA1/2* so further genes remain to be discovered.
- Due to founder effects, certain ethnic groups such as the Ashkenazi population has a higher chance of having specific mutations.
- There is some early evidence that certain factors (e.g. hormonal manipulation) may modify penetrance.
- The breast cancer risk burden from low penetrance genes in the whole population could be high, but this is uncertain.
- Lifetime (by the age of 80 years) cancer risks in *BRCA1/2* carriers:

	Cancer type				
Gene	Female breast	Ovarian	Male breast	Colon	Prostate
BRCA1	80–85%	60%	?0	6%	6%
BRCA2	80–85%	27%	5%	?0	6–14%

The use of genetic information by insurance companies is a highly contentious issue. One view is that genetic tests are like any other medical test and medical test results have to be declared when seeking insurance. Conversely, there is a fear that genetic test results used for insurance purposes may create an uninsurable underclass. The whole principle of insurance is to insure the individual by encompassing the risk of the entire pool and the effect of improvements in genetic information will be to define more clearly which different risks individuals carry within the pool. It is possible that genetic test results may be ignored for the purposes of insurance; this is already the position taken by some insurance companies and is the position in some European countries (e.g. The Netherlands [71]).

The advances in knowledge about genetic predisposition to common cancers that will accompany the completion of the Human Genome Project will bring exciting developments, both for the scientist and for clinicians managing individuals with a genetic predisposition to cancer. It will facilitate targeted screening with better screening measures, and the improvements in technology and knowledge about the complete human genetic sequence will increase the speed of genetic testing. Testing will be able to be offered to individuals who currently cannot be offered a test, either because they are unaffected in a family where all affected members cannot be tested because they are deceased, or because the gene that predisposes to the cancer in their family is currently not identified. However, these advances will be accompanied by profound issues for society. The ethical, legal and social implication programme accompanying the Human Genome Project will be a very important component of this programme. The current status of genetic predisposition to breast cancer and a futuristic look are summarised in summary boxes 1 and 2 The face of medicine will be completely changed by the middle of the next millennium. Emphasis will be on the genetic classification of disease. There will be a more proactive style of practising medicine to identify risks of disease before symptoms develop, enabling early detection and preventive measures to be taken.

Summary Box 2
Genetic Predisposition to Breast Cancer: Predictions for the Next Millennium

- Approximately 86% of breast cancer is due to some genetic predisposing component.
- 5–10% is due to high-risk genes.
- The rest is due to moderate–low-risk genes.
- All the breast cancer genetic risk sequences are known.
- Unaffected individuals can be tested directly for a 'breast cancer risk profile'.
- Drugs are available to prevent breast cancer in both high- and low-risk groups.
- There are better methods of earlier detection.
- The ethical, legal and social issues of pre-implantation/prenatal testing, large-scale genetic profiling and insurance will have been addressed.

1. Office for National Statistics series DH2.NO23. *Mortality Statistics by Cause—England and Wales 1996*. London, Her Majesty's Stationery Office, 1998.
2. Broca PP. *Traites des Tumeurs*. Paris, Asselin, 1866.
3. Easton D, Peto J. The contribution of inherited predisposition to cancer incidence. *Cancer Surveys* 1990, **9**, 395–416.
4. Claus EB, Risch NJ, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. *Am J Epidemiol* 1990, **131**, 961–972.
5. Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet* 1991, **48**, 232–242.
6. Hall JM, Lee MK, Newman B, *et al*. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990, **250**, 1684–1689.
7. Narod SA, Feunteun J, Lynch HT, *et al*. Familial breast–ovarian cancer locus on chromosome 17q12-q23. *Lancet* 1991, **338**, 82–83.
8. Miki Y, Swensen J, Shattuck-Eidens D, *et al*. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* 1994, **266**, 66–71.
9. Easton DF, Bishop DT, Ford D, *et al*. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 1993, **52**, 678–701.
10. Ford D, Easton DF, Stratton M, *et al*. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *The Breast Cancer Linkage Consortium Am J Hum Genet* 1998, **62**, 676–689.
11. Wooster R, Bignell G, Lancaster J, *et al*. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995, **378**, 789–792. (published erratum appears in *Nature* 1996, Feb **22**, 379(6567):749).
12. Easton DF. From families to chromosomes: genetic linkage, and other methods for finding cancer-predisposition genes. In Eeles RA, Ponder BAJ, Easton DF, Horwich A, eds. *Genetic Predisposition to Cancer*. London, Chapman & Hall Medical, 1996, 16–39.
13. Shattuck-Eidens D, McClure M, Simard J, *et al*. A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening. *J Am Med Assoc* 1995, **273**, 535–541.
14. Shattuck-Eidens D, Oliphant A, McClure M, *et al*. *BRCA1* sequence analysis in women at high risk for susceptibility mutations. Risk factor analysis and implications for genetic testing. *J Am Med Assoc* 1997, **278**, 1242–1250.
15. BIC (Breast Cancer Information Core) database, accessible on the internet at http://www.nchgr.nih.gov/dir/lab_transfer/bic/
16. Smith SA, Easton DF, Evans DG, *et al*. Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. *Nature Genet* 1992, **2**, 128–131.
17. BIC (Breast Cancer Information Core) database, accessible on the internet at http://www.nchgr. nih.gov/dir/lab_transfer/bic/
18. Ford D, Easton DF, Peto J. Estimates of the gene frequency of *BRCA1* and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet* 1995, **57**, 1457–1462.
19. FitzGerald MG, MacDonald DJ, Krainer M, *et al*. Germ-line *BRCA1* mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med* 1996, **334**, 143–149.

20. Langston AA, Malone KE, Thompson JD, *et al*. *BRCA1* mutations in a population-based sample of young women with breast cancer. *N Engl J Med* 1996, **334**, 137–142.
21. Struewing JP, Abeliovich D, Peretz T, *et al*. The carrier frequency of the *BRCA1* 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nature Genet* 1995, **11**, 198–200.
22. Offit K, Gilewski T, McGuire P, *et al*. Germline *BRCA1* 185delAG mutations in Jewish women with breast cancer. *Lancet* 1996, **347**, 1643–1645.
23. Tonin P, Weber B, Offit K, *et al*. Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. *Nature Med* 1996, **2**, 1179–1183.
24. Roa BB, Boyd AA, Volcik K, *et al*. Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2*. *Nature Genet* 1996, **14**, 185–187.
25. Oddoux C, Struewing JP, Clayton CM, *et al*. The carrier frequency of the *BRCA2* 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nature Genet* 1996, **14**, 188–190.
26. Neuhausen S, Gilewski T, Norton L, *et al*. Recurrent *BRCA2* 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nature Genet* 1996, **13**, 126–128.
27. Ford D, Easton DF, Bishop DT, *et al*. Risks of cancer in *BRCA1*-mutation carriers. *Breast Cancer Linkage Consortium Lancet* 1994, **343**, 692–695.
28. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in *BRCA1*-mutation carriers. Breast Cancer Linkage Consortium. *Am J Hum Genet* 1995, **56**, 265–271.
29. Goldgar DE, Fields P, Lewis CM, *et al*. A large kindred with 17q-linked breast and ovarian cancer: genetic, phenotypic and geneological analysis. *J Natl Cancer Inst* 1994, **86**, 200–209.
30. Gayther SA, Warren W, Mazoyer S, *et al*. Germline mutations of the *BRCA1* gene in breast and ovarian cancer families provide evidence for a genotype–phenotype correlation. *Nature Genet* 1995, **11**, 428–433.
31. Phelan CM, Lancaster JM, Tonin P, *et al*. Mutation analysis of the *BRCA2* gene in 49 site-specific breast cancer families. *Nature Genet* 1996, **13**, 120–122.
32. The Breast Cancer Linkage Consortium. Cancer risks in *BRCA*2 mutation carriers. *J Natl Cancer Inst* 1999, **91**, 1310–1316.
33. Craufurd D, Dodge A, Kerzin SL, *et al*. Uptake of presymptomatic predictive testing for Huntington's disease. *Lancet* 1989, **2**, 603–605.
34. Craufurd D, Evans DGR, Binchy A. Response to *BRCA1* (linkage) testing. Poster presented to the Cancer Family Study Group. 1995, Manchester, U.K.
35. Watson M, Murday V, Lloyd S, *et al*. Genetic testing in breast/ovarian cancer (*BRCA1*) families. *Lancet* 1995, **346**, 583.
36. Watson M, Lloyd SM, Eeles RA, *et al*. Psychosocial impact of testing (by linkage) for the *BRCA1* breast cancer gene: an investigation of two families in the research setting. *Psycho-Oncology* 1996, **5**, 233–239.
37. Struewing JP, Hartge P, Wacholder S, *et al*. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N Engl J Med* 1997, **336**, 1401–1408.
38. Thorlacius S, Struewing JP, Hartge P, *et al*. Population-based study of risk of breast cancer in carriers of *BRCA2* mutation. *Lancet* 1998, **35**, 1337–1339.
39. Chamberlain J. Screening for breast cancer in high-risk populations. In Eeles RA, Ponder BAJ, Easton DF, Horwich A, eds. *Genetic Predisposition to Cancer*. London, Chapman & Hall Medical, 1996, 253–266.
40. Eccles D, Evans DGR, MacKay J, *et al*. Guidelines for a genetic risk-based approach to advising women with a family history of breast cancer. *J Med Genet* 1999, in press.
41. Lalloo F, Boggis CRM, Evans DGR, *et al*. Screening by mammography, women with a family history of breast cancer. *Eur J Cancer* 1998, **34**, 937–940.
42. MRI Breast Screening Study Advisory Group. National study of magnetic-resonance imaging to screen women at genetic risk of breast cancer. *Lancet Interactive*, Protocol Reviews, 1998, Protocol P7/4 (http://www.thelancet.com/newlancet/reg/author//protocol7_4.html).
43. Mitchell G, Trott PA, Coleman N, et al. Nipple fluid aspiration (NFA): a ''PAP smear'' for the breast in *BRCA1/2* carriers? *Am J Hum Genet* 1999, **65**(Suppl.), A311 (abstract no. 1748).
44. Gudas JM, Nguyen H, Li T, *et al*. Hormone-dependent regulation of BRCA1 in human breast cancer cells. *Cancer Res* 1995, **55**, 4561–4565.
45. Marquis ST, Rajan JV, Wynshaw BA, *et al*. The developmental pattern of *BRCA1* expression implies a role in differentiation of the breast and other tissues. *Nature Genet* 1995, **11**, 17–26.
46. Narod SA, Goldgar D, Cannon-Albright L, *et al*. Risk modifiers in carriers of *BRCA1* mutations. *Int J Cancer* 1995, **64**, 394–398.
47. Narod SA, Risch H, Moslehi R, *et al*. Oral contraceptives and the risk of hereditary ovarian cancer. *N Engl J Med* 1998, **339**, 424–428.
48. Ursin G, Henderson BE, Haile RW, *et al*. Does oral contraceptive use increase the risk of breast cancer in women with *BRCA1*/*BRCA2* mutations more than in other women? *Cancer Res* 1997, **57**, 3678–3681.
49. Brunet JS, Ghadirian P, Rebbeck TR, *et al*. Effect of smoking on breast cancer in carriers of mutant *BRCA1* or *BRCA2* genes. *J Natl Cancer Inst* 1998, **90**, 761–765.
50. Rebbeck T, *et al*. Breast cancer risk after bilateral prophylactic oophorectomy in *BRCA1* mutation carriers. *J Natl Cancer Inst* 1999, **91**, 1475–1479.
51. Powles T, Eeles R, Ashley S, *et al*. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet* 1998, **352**, 98–101.
52. Fisher B, Costantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998, **90**, 1371–1388.
53. Veronesi U, Maisonneuve P, Costa A, *et al*. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. Italian Tamoxifen Prevention Study. *Lancet* 1998, **352**, 93–97.
54. Hartmann LC, Schaid DJ, Woods JE, *et al*. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. *N Engl J Med* 1999, **340**, 77–84.
55. Struewing JP, Hartge P, Wacholder S, *et al*. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N Engl J Med* 1997, **336**, 1401–1408.
56. Tobacman JK, Greene MH, Tucker MA, *et al*. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian-cancer-prone families. *Lancet* 1982, **2**, 795–797.
57. Eltabbakh GH, Piver MS. Extraovarian primary peritoneal carcinoma. *Oncol Huntingt* 1998, **12**, 813–819.
58. Feigelson HS, Coetzee GA, Kolonel LN, *et al*. A polymorphism in the *CYP17* gene increases the risk of breast cancer. *Cancer Res* 1997, **57**, 1063–1065.
59. The Chipping Forecast. *Nature Genet* 1999, **21**.
60. Kote-Jarai Z. Eeles RA. BRCA1 and BRCA2 in a common pathway in DNA damage response. *Br J Cancer* 1999, in press.
61. Milner J, Ponder B, Hughes DL, *et al*. Transcriptional activation functions in BRCA2. *Nature* 1997, **386**, 772–773.
62. Chapman MS, Verma IM. Transcriptional activation by *BRCA1*. *Nature* 1996, **382**, 678–679.
63. Humphrey JS, Salim A, Erdos MR, *et al*. Human *BRCA1* inhibits growth in yeast: potential use in diagnostic testing. *Proc Natl Acad Sci USA* 1997, **94**, 5820–5825.
64. Lerman C, Narod S, Schulman K, *et al*. *BRCA1* testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. *J Am Med Assoc* 1996, **275**, 1885–1892.
65. Lerman C, Daly M, Masny A, *et al*. Attitudes about genetic testing for breast-ovarian cancer susceptibility. *J Clin Oncol* 1994, **12**, 843–850.
66. Watson M, Lloyd SM, Eeles RA, *et al*. Psychosocial impact of testing (by linkage) for the *BRCA1* breast cancer gene: an investigation of two families in the research setting. *Psychol Oncol* 1996, **5**, 233–239.
67. Watson M, Lloyd S, Davidson J, *et al*. The impact of genetic counselling on risk perception and mental health in women with a family history of breast cancer. *Br J Cancer* 1999, **79**, 868–874.
68. Tops CM, Wijnen JT, Griffioen G, *et al*. Presymptomatic diagnosis of familial adenomatous polyposis by bridging DNA markers. *Lancet* 1989, **2**, 1361.

69. Lallier M, St VD, Giroux M, *et al*. Prophylactic thyroidectomy for medullary thyroid carcinoma in gene carriers of MEN2 syndrome. *J Pediatr Surg* 1998, **33**, 846–848.
70. Ao A, Wells D, Handyside AH, *et al*. Preimplantation genetic diagnosis of inherited cancer: familial adenomatous polyposis coli. *J Assist Reproduct Genet* 1998, **15**, 140–144.
71. Morrison PJ, Steel CM, Evans DGR, *et al*. Breast cancer testing and insurance guidelines in Europe: the need for legislation? *Biomed 2: Familial Breast Cancer Demonstration Project*, published at website http://www.abdn.ac.uk/~mmd249/Abstract.html