Part 1

Molecular biology and natural history

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Introduction

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It has long been recognized that some very rare forms of cancer, such as retinoblastoma and neurofibromatosis, are caused by inherited genes. It is only within the last few years, however, that rapid progress has been made in understanding the role that inherited genes also play in determining a proportion of the more common cancers, including breast, colorectal and ovarian cancer. Although there is still uncertainty about the precise contribution of inherited predisposition genes to the incidence of these cancers, the available evidence suggests that breast, colorectal and ovarian cancer have a number of common genetic features.

- A small proportion of these cancers (about 5%) are caused by inherited genes which, though comparatively rare, confer very high lifetime risks of developing cancer. In some cases these lifetime risks may be as high as 80%.
- Cancers caused by these high penetrance genes are more likely to occur at an early age than sporadic cancers, and 15–20% of the cancers diagnosed in people under the age of 50 years may be accounted for by these genetic mutations.
- Carriers of known genetic mutations, which confer high lifetime risks of developing breast or ovarian cancer, are also at significantly increased risk of developing certain other forms of cancer.
- A further 10–20% of breast, ovarian and colorectal cancers are likely to be caused by inherited polymorphisms in predisposition genes, which are commoner but less penetrant but which confer some increased risk (more than three times the general population risk). These 'medium risk' genes have not yet been clearly identified.
- Familial clustering of the more common cancers may also be influenced by environmental and lifestyle factors as well as by chance.

Services for cancer genetics

Cancer genetics is a new field and the organization of services in this area may be initiated by clinical genetics services or through oncology and other departments,

where individuals with a special interest in cancer genetics arrange to see individuals with a family history of cancer, estimate their cancer risks and arrange surveillance and genetic testing as appropriate.

In many parts of Europe, cancer genetics clinics have been established for many years, and most specialized genetic counselling for cancer susceptibility is organized from genetics centres. However, the organization and quality of such services vary, depending on the economic status and healthcare systems of the country. There is increasing awareness that education and referral guidelines for primary care physicians are important. This would allow a collaborative relationship to be developed with primary healthcare services, helping them act as gatekeepers for the prioritization of referrals for genetics services.

Growing public awareness of the familial risks of cancer has led to a rapid increase in demand for advice about these risks and in the number of referrals to genetics clinics in all parts of Europe. Many of these clinics lack the resources to meet this demand and as a result of this and a desire to provide the 'best' service possible to high-risk patients, clinics need to ensure that the service they provide is evidence based. Where evidence is lacking, an audit of 'best practice' guidelines is essential to provide the information. In order to obtain sufficient information, a very large cohort of at-risk individuals, well documented for family history, needs to be followed up for many years. Such an audit is only possible with large multicentre trials, and the emerging European and North American collaborations are an ideal forum for this.

The basic aims of genetic services for people concerned about familial risks of breast or ovarian cancer are: (1) to provide advice and counselling about familial risks, and (2) to identify those who are at an increased risk. Where possible, molecular tests for mutations in genes such as *BRCA1* and *BRCA2* may be possible, and predictive testing will allow the identification of very-high-risk individuals. Once identified, these individuals will need to be enrolled in effective protocols for the management of their risk and for the treatment of cancers detected. Large-scale evaluation of such management is facilitated by European collaborative studies.

This collection of chapters sets out guidelines for assessing whether individuals are at an increased risk of developing cancer on the basis of their family history. The initial point of contact for many individuals concerned about familial cancer risks is the family doctor. Hence guidelines could be provided to family doctors and their staff to assist them in the assessment of risks. Individuals considered to be at sufficiently high risk could be referred to genetics clinics, where a more detailed assessment would be carried out.

Individuals who are assessed as being at an increased risk of developing cancer, but often where no *BRCA1* or *BRCA2* mutation can be identified, should be

offered regular screening. Suggested screening protocols for the management of these individuals, covering the age range, frequency and type of screening, are outlined in chapters benefiting from the collective experience of groups from many European countries and from the USA and Canada.

Cancer genetics is an area where medical knowledge is developing rapidly, and there will be a continuing need to assess the implications of new research into the genetic aspects of breast and ovarian cancer for the screening programmes currently recommended. The implications of research into the genetic aspects of other forms of cancer may also need to be assessed. The benefit of the experience of different countries and cultures in implementing the health strategies suggested by the results of such research is important.

Eleven centres in Europe worked together from 1997 on an EU-funded demonstration project entitled: 'Familial Breast Cancer: Audit of a New Development in Medical Practice in European Centres'.

The chapters included in this book were generated, in part, from the work of this project and provide guidelines, evidence and consensus views for a variety of aspects of patient care within a cancer genetics clinic. The other chapters originate from colleagues worldwide who are providing evidence in other areas of cancer genetics on which patient care can be based.

As a final outcome of the EU demonstration project, the 'International Collaborative Group on Familial Breast and Ovarian Cancer' was established. This worldwide group will continue the work of the project and will, in addition, collaborate and integrate with other groups and individuals who share a common interest in producing evidence to develop our understanding of the inherited cancers and hence improve the care of patients and their families.

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Overview of the clinical genetics of breast cancer

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Introduction

Breast cancer is the most common cancer in women, accounting for 20% of all new cases of cancer. The lifetime risk to a woman in the UK is 1 in 12 females, with an incidence of less than 10 per 100 000 women aged under 30 years, rising to 300 per 100 000 in women aged over 85 years. Breast cancer can occur in sporadic and hereditary forms, and both forms are associated with modification to the genetic material. In the case of hereditary forms, a constitutive mutation in a specific gene predisposes individuals to cancer. In sporadic forms, mutations in somatic cells accumulate and result in transformation of a normal cell to one with malignant potential.

Statistical analysis of epidemiological data is compatible with a dominant gene (or genes) predisposing to breast cancer in certain families, with 5–10% of breast cancer being due to highly penetrant, dominant genes (Easton and Peto, 1990; Claus et al., 1991). Approximately 10% of isolated cases presenting under 35 years may be due to such a gene but only 1% of cases presenting over 80 years (Langston et al., 1996; Ford et al., 1998).

Table 2.1 lists genetic syndromes that may predispose to breast cancer, some of which will be discussed in this chapter and in some greater detail in subsequent chapters.

Family history as an indicator of predisposition to breast cancer

A history of breast cancer among relatives has been found, in epidemiological studies, to be an indication of breast cancer risk. Familial breast cancer is characterized by: a younger age at diagnosis than sporadic forms, increasing numbers of affected family members, an increased risk of bilateral breast cancer, and a strong association with ovarian cancer.

Table 2.1. Genetic syndromes associated with breast cancer susceptibility

Syndrome	Gene/chromosome
1. Site-specific hereditary breast cancer	*BRCA1*, *BRCA2*, +
2. Breast/ovarian cancer	*BRCA1*, *BRCA2*
3. Li–Fraumeni syndrome	*TP53*
4. Ataxia-telangiectasia syndrome	*ATM*
5. Cowden disease	*PTEN*
6. Klinefelter's syndrome	47, XXY
7. Muir–Torre syndrome	*MSH2*, *MLH1*
8. Peutz–Jeghers syndrome	*STK11/LKB1*

If a woman has a first-degree relative (mother, sister or daughter) who has developed breast cancer before the age of 50 years, her own risk of developing the disease is increased two-fold or greater, and the younger the relative the greater is the risk. If a woman has two first-degree relatives with the disease, her risk may be increased four- to six-fold, and again, the younger the relative the greater is the risk (Claus et al., 1996; McPherson et al., 2000). It must also be remembered that males can pass on genes predisposing to breast cancer and hence it is also relevant to consider the history of breast cancer in female relatives of the father of a consultand.

Studies of familial breast cancer

It has been recognized for many years that there is an association in certain families between breast and ovarian cancer. The risk for epithelial ovarian cancer was found to be significantly elevated in patients with first-degree relatives affected with breast cancer (twice the population risk) (Muderspach, 1994; Claus et al., 1996). Similarly, the risk for breast cancer was found to be elevated in patients who had first-degree relatives with ovarian cancer.

Following international studies of large families with an excess of both early-onset breast cancer and of ovarian cancers, Mary Clair King's group demonstrated linkage between inherited susceptibility to early-onset breast cancer and a polymorphic marker on chromosome 17q21.3 (Hall et al., 1990). Predisposition to breast and ovarian cancer was also found with this locus in many families around the world, but it was also clear that other families existed with an excess of early-onset breast cancer that did not segregate with this locus (Narod et al., 1995). Subsequently, by studying, among others, families with male and female breast cancer, a second locus was found on chromosome 13q12–13 (Wooster et al., 1994).

It is now clear that there are other families with a predominance of breast cancer who are not linked to either of these loci, and hence it is likely that other genes exist that predispose individuals to breast cancer (Bishop, 1994).

Molecular features

Following the linkage studies, two genes were identified that are implicated in the pathogenesis of breast and ovarian cancer: *BRCA1* localized to chromosome 17q21 (Hall et al., 1990; Miki et al., 1994) and *BRCA2* localized to chromosome 13q12–13 (Wooster et al., 1994). These two genes would appear to account for almost all families with breast and ovarian cancer predisposition and also for approximately 50% of families with predisposition to breast cancer alone (Ford et al., 1994).

Genes implicated in breast cancer predisposition

BRCA1

The *BRCA1* gene on chromosome 17q21 was identified by positional cloning methods and found to have 5592 coding nucleotides that are distributed over 100 000 bases of genomic DNA and has 22 coding exons. These encode a protein of 1863 amino acids. Loss of the wild-type allele was found in over 90% of tumours from women with a germline mutation in *BRCA1*, and hence it is regarded as a tumour suppressor gene. In addition, transfection of wild-type *BRCA1* into breast/ovarian cell lines decreased cell proliferation, whereas mutant *BRCA1* did not (Koonin et al., 1996).

Sequence analysis of *BRCA1* indicates that it has a C3HC4 zinc-binding RING-finger domain at the amino terminus of the protein, a classic simian virus 40-type nuclear localization sequence in exon 11, and two regions resembling the transactivating domain of a number of transactivation factors called '*BRCT*' (*BRCA1* carboxyl-terminal) domains. Other proteins with similar domains function in cell cycle control and DNA damage repair pathways (Bork et al., 1997; Callebaut and Mornon, 1997).

Mutations

More than 500 sequence variations have been identified in *BRCA1*, and of these, more than 80% of all *BRCA1* mutations are frameshift or nonsense mutations that alter the codon reading frame and result in a 'stop' codon producing a premature protein termination (Futreal et al., 1994; Gayther et al., 1995; FitzGerald et al., 1996; Szabo and King, 1997; Liede et al., 1999). Genetic susceptibility to breast cancer is thought to occur when one *BRCA1* allele is inactivated in the germline

Table 2.2. Prevalence of *BRCA1* and *BRCA2* genes

	Gene mutation	General population (%)	Breast cancer (%)	Age group (years)
Ashkenazi	*BRCA1:* 185del AG	0.8	20	<42
	BRCA1: 5382insC	0.4		
	BRCA2: 6174delT	1.2	8	<42
Icelandic	*BRCA2:* 999del5	0.6	24	<40
British	*BRCA1:* all mutations	0.11	3.1	<49
	BRCA2: all mutations	0.12	3.0	<49

From Peto et al. (1999); Johannesdottir et al. (1996); Struewing et al. (1997).

and subsequently the other allele is lost in somatic breast tissue. The most common mutations, so far discovered, are 185delAG and 5382insC (Table 2.2). In addition, germline deletions have been found, and may be associated with the high frequency of 'Alu' repeats in the introns. In the Dutch/Belgium population, three large deletions have been identified and account for 30% of all germline mutations in Dutch families. Four novel deletions have recently been found in the regulatory regions of the *BRCA1* gene in French and American families (Peelen et al., 1997; Petrij-Bosch et al., 1997; Puget et al., 1999a, 1999b).

Penetrance and prevalence

Collaborative studies by the Breast Cancer Linkage Consortium (BCLC) have examined multiple families with germline mutations in *BRCA1* and *BRCA2* to establish the penetrance of mutations in these genes and the risks of other cancers (Ford et al., 1994; Ford et al., 1998; Puget et al., 1999a) (Figure 2.1). These studies suggest that carriers of mutations in *BRCA1* have an associated cumulative breast cancer risk of 80–85% by age 80 years. Once affected with a first breast cancer, such gene carriers have a subsequent risk of contralateral breast cancer estimated to be up to 48% by age 50 years and 64% by age 70 years. Similarly, the risk of ovarian cancer in carrier women is 60% by age 80 years as compared with a population risk of around 1%. Colon cancer risk is increased to 6% by the age of 70 years and prostate cancer may occur three times more often than expected in male *BRCA1* mutation carriers, with an absolute risk of 6% by age 74 years (Ford et al., 1998).

There is a correlation between the position of the mutation within the gene and the ratio of breast to ovarian cancer incidence in a family. It has been noted that mutations in the 3′ third of the gene are associated with a lower proportion of ovarian cancer (Futreal et al., 1994), although it is not known whether this is due

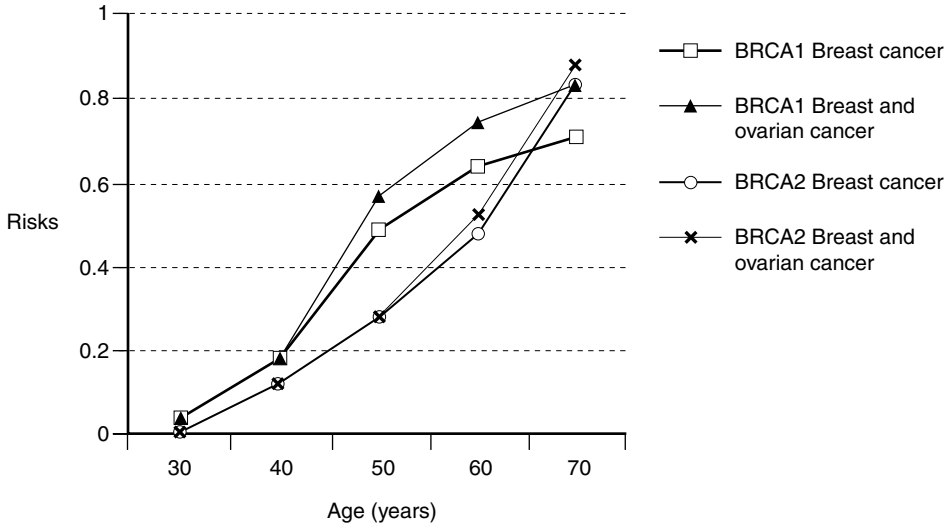

Figure 2.1 Breast cancer and breast and ovarian cancer risks in *BRCA1* and *BRCA2* mutation carriers (Breast Cancer Linkage Consortium, 1999).

to a difference in penetration of the mutation for breast cancer, ovarian cancer or both (Gayther et al., 1995; Rahman and Stratton, 1998).

Germline mutations in *BRCA1* account for 15–45% of hereditary breast cancer and around 80% of breast/ovarian cancer families (Table 2.3). In addition, as is seen in Table 2.4, there is evidence for an increased risk of colorectal and prostate cancer (Ford et al., 1998). Studies suggest that *BRCA1* accounts for about 1% of breast cancer in the general population (Peto et al., 1999) but about 3% of those breast cancers occurring in women aged less than 49 years and 0.49% of women aged more than 50 years (Table 2.5).

BRCA2

A second breast cancer susceptibility gene (*BRCA2*) was localized to chromosome 13q12–13 (Wooster et al., 1994). In these families, cases of male breast cancer were found to be a part of the *BRCA2* tumour spectrum, and in addition, the risk of ovarian cancer is lower than in families with *BRCA1*.

BRCA2 was cloned and found to be a large gene (Easton et al., 1995; Wooster et al., 1995). It has 11 385 coding nucleotides that are distributed over 70 000 bases of genomic DNA and has 27 exons coding for a protein of 3418 amino acids. It bears no clear homology to previously described genes and its protein has no previously defined functional domains. Eight copies of a 30–80 amino acid repeat (BRC repeat) are coded in the portion of the protein encoded by exon 11. These domains are highly conserved and are postulated to be involved in the binding of *RAD51* to *BRCA2* protein (Callebaut and Mornon, 1997).

Table 2.3. Heterogeneity analysis

	Family type	No. of families	*BRCA1*	*BRCA2*	Other
Families with female breast cancer only (no ovarian cancer or male breast cancer)	All	117	0.28	0.37	0.35
	6+ breast cancers	34	0.19	0.66	0.15
	4–5 breast cancers	83	0.32	0.09	0.59
Families with breast/ovarian cancer	All	94	0.80	0.15	0.05
	2+ ovarian cancers	52	0.88	0.12	0.00
	1 ovarian cancer	42	0.69	0.19	0.12
Families with at least one male breast cancer	All	26	0.19	0.77	0.04
All families	All	237	0.52	0.35	0.13
	6+ breast cancers	83	0.46	0.50	0.04
	4–5 breast cancers	154	0.55	0.12	0.33

From Ford et al. (1998).

Table 2.4. Risks of cancers other than breast/ovarian cancers in *BRCA1* and *BRCA2* mutation carriers

	Cancer type	Relative risk
BRCA1	Colon	3.30
	Prostate	4.11
BRCA2	Stomach	2.59
	Pancreas	3.51
	Gallbladder	4.97
	Melanoma	2.58
	Prostate	4.65

From Ford et al. (1998); Breast Cancer Linkage Consortium (1999).

Mutations

Like *BRCA1*, more than 250 distinct mutations in *BRCA2* have been identified, scattered throughout this gene. To date, insufficient evidence is available for the risk associated with most missense mutations to be calculated with certainty and