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Breast Cancer Families in Finland

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Academic Dissertation

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Contents

Abbreviations

95% CI	95% confidence interval
ASO	allele-specific oligonucleotide
ATM	ataxia telangiectasia mutation
BCLC	Breast Cancer Linkage Consortium
BIC	Breast Cancer Information Core
BRCA1	breast cancer gene 1
BRCA2	breast cancer gene 2
CHK2	checkpoint kinase 2
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
ERBB2	avian erythroblastic leukaemia viral oncogene
FCR	Finnish Cancer Registry
HA/SSCP	heteroduplex analysis/single strand conformation polymorphism
HNPCC	hereditary non-polyposis colorectal cancer
LOH	loss of heterozygosity
MEN	multiple endocrine neoplasia
OCCR	ovarian cancer cluster region
PID	personal identifier
PTEN	phosphatase and tensin homolog
PSA	prostate-specific antigen
PTT	protein truncation test
RET	ret proto-oncogene
RFLP	restriction fragment-length polymorphism
RR	relative risk
RR	relative excess risk of death (Study IV)
RSR	relative survival rate
SIR	standardized incidence ratio
SMR	standardized mortality ratio
STK11	serine-threonine kinase 11

List of Original Publications

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals:

I. Eerola H, Blomqvist C, Pukkala E, Pyrhönen S, Nevanlinna H. Familial breast cancer in southern Finland: how prevalent are breast cancer families and can we trust the family history reported by the patients? Eur J Cancer, 36: 1143-1148, 2000

II. Muhonen T, Eerola H, Vehmanen P, Nevanlinna H, Aktan K, Blomqvist C, Kääriäinen H, Pyrhönen S. Breast cancer risk estimation in families with history of breast cancer. Br J Cancer, 76: 1228-1231, 1997

III. Eerola H, Pukkala E, Pyrhönen S, Blomqvist C, Sankila R, Nevanlinna H. Risk of cancer in BRCA1 and BRCA2 mutation-positive and -negative breast cancer families. Cancer Causes Contr, 12: 739-746, 2001

IV. Eerola H, Vahteristo P, Sarantaus L, Kyyrönen P, Pyrhönen S, Blomqvist C, Pukkala E, Nevanlinna H, Sankila R. Survival of breast cancer patients in BRCA1, BRCA2, and non-BRCA1/2 breast cancer families: a relative survival analysis from Finland. Int J Cancer, 93: 368-372, 2001

Abstract

Breast cancer is the most common malignancy among women worldwide. Hereditary disposition is estimated to cause about 7 % of breast cancer. This thesis evaluates family history of breast and ovarian cancer among breast cancer patients and describes identification of breast cancer families in Finland. In addition, we studied the accuracy of the patients' reports on family history, risk of cancer, and survival of the breast cancer patients in the identified families.

Family history of cancer was screened for among breast cancer patients in the Department of Oncology, Helsinki University Central Hospital. Genetic susceptibility to breast cancer is closely associated with susceptibility to ovarian cancer, and we defined breast cancer families by the selection criterion of at least three first- or second-degree relatives with breast or ovarian cancer (including the proband, i.e., the index patient originally interviewed). We confirmed the genealogy of the families through population registries and cancer diagnoses through the Finnish Cancer Registry.

In Study I, family history of breast cancer appeared to be relatively common, as about 30% of the patients reported at least one first- or second-degree relative with breast or ovarian cancer. Our criterion identified 7 to 9% of the patients into breast cancer families. Comparison of the information reported by patients to that obtained from registries showed that patients were well aware of their affected relatives. They reported 87% of all confirmed diagnoses, the primary site being correct in 93 to 95% of the cases they reported. Although the incompleteness and errors in accuracy were small, these should be taken into account in epidemiological studies, and verification of the diagnoses of the relatives is important when deciding on clinical management.

While accurate family history is critical in finding breast cancer families, their identification is not straightforward. Breast cancer is a common disease, and multiple cases may be clustered in families only by chance. We evaluated the standard life-table method for identification of high-risk families in a series of families with the proband diagnosed either as under 40 years or with bilateral disease (II). The risk of breast cancer among all first-degree relatives was about 7.6–fold greater than the risk in the general

population. The method takes into account all family members as well as their age at onset. The life-table model could provide a useful tool for selecting individuals for genetic counselling or surveillance.

BRCA1 and BRCA2 germ line mutations predispose to a high risk of breast and ovarian cancer, and elevated risks for other cancers have been reported as well. However, the risk of cancer has not been evaluated in the mutation-negative or in the Finnish BRCA1/2 mutation-positive breast cancer families previously. The standardized incidence ratio (SIR) of ovarian cancer for first-degree relatives of breast cancer patients was very high in BRCA1 (SIR 29, 95% confidence interval 9.4-68) and in BRCA2 families (SIR 18, 8.4-35), but was not increased in first-degree relatives of breast cancer patients in BRCA1/2 mutation-negative families (SIR 1.0, 0.2-2.9). The risk of breast cancer in all three groups was noticeably increased. The risk of subsequent cancers of the breast and ovary was high among breast cancer patients in BRCA1 and BRCA2 families, and the risk of subsequent breast cancer was high among breast cancer patients in mutation-negative families. The only elevated SIR, besides that for breast and ovarian cancer, was that for prostate cancer in BRCA2 families. The excess risk of breast cancer in non-BRCA1/2 families suggests existence of other susceptibility genes which seem not to predispose to ovarian cancer (III).

Family history of breast cancer has been associated with increased, similar, and decreased survival among breast cancer patients as compared to breast cancer patients in general. We found no significant difference in survival of breast cancer patients between BRCA1 or BRCA2 or BRCA1/2 mutation-negative families, and the general population. Based on this and previous studies, it is probable that no large prognostic differences exist (IV).

Women predisposed to hereditary or familial breast cancer form a heterogeneous group. Having a family history of the disease is relatively common among breast cancer patients, but only a minority belongs to families with a true disposition to cancer. All estimations of the risk of cancer should be based on an accurate family history and, if possible, on mutation screening. Surveillance of both breast and ovarian cancers is needed for the carriers of BRCA1 and BRCA2 mutations. The risk of subsequent cancers of the breast and ovary is high, and it should also be noted in treatment and follow-up decisions. Whereas males among BRCA2 families may have an increased risk of prostate cancer,

risk of other cancers than breast, ovary, and prostate do not appear to be sufficiently increased to warrant special follow-up programmes in any of these three groups of families. Families without BRCA1 or BRCA2 mutations also show an increased risk of breast cancer, and in future, other susceptibility genes are likely to appear; breast cancer surveillance is necessary also in those families. More studies are urgently needed to clarify the risk of cancer, patient survival, efficacy of surveillance, and management of patients and healthy relatives.

Introduction

Breast cancer is the most common malignancy affecting women worldwide (Ferlay et al., 2001), and in Finland, 3 324 new breast cancer cases were diagnosed in 1997 (Finnish Cancer Registry, 2000). One of the strongest risk factors for breast cancer is family history (Madigan et al., 1995). As breast cancer is a common disease and multiple cases may occur in a family by chance, it is not easy to distinguish hereditary cases from those occurring in the family by coincidence. However, accurate and detailed family history forms the basis of identification. It has been estimated that 7% of breast cancer is due to hereditary predisposition (Claus et al., 1996). Two major dominantly inherited genes predisposing to breast and ovarian cancer have been identified, BRCA1 (Miki et al., 1994) and BRCA2 (Wooster et al., 1995), with high risk of early-onset breast cancer and varying risk of ovarian cancer. The risk of some other cancers, like prostate and colon, has been documented to be elevated in the families with identified BRCA1/2 mutations or linkage to the BRCA1 or BRCA2 gene (Ford et al., 1994; Breast Cancer Linkage Consortium, 1999). However, the cancer risks in BRCA1 and BRCA2 breast cancer families have been variable and mostly based either on highly selected families or on individual mutations in founder populations (Ford et al., 1994; Struewing et al., 1997; Thorlacius et al., 1998; Breast Cancer Linkage Consortium, 1999). The risk of cancer has not been previously investigated among Finnish BRCA1 and BRCA2 families. Furthermore, in a large proportion of breast cancer families, the predisposition is not explained by these two genes, instead the cancer appears to be caused by other genes presently unknown (Vehmanen et al., 1997 a; Vehmanen et al., 1997 b; Ford et al., 1998; Peto et al., 1999). In these BRCA1/2 mutation-negative families, the risk of cancer has not been previously documented. Reports on the prognosis of familial breast cancer have been contradictory (reviewed in Chappuis et al., 1999; Phillips et al., 1999). If true differences in survival exist, they would have important implications for genetic counselling and in treatment of hereditary breast cancer cases.

Review of the literature

General features of breast cancer

Breast cancer is the most common cancer affecting women in Finland. About one in ten Finnish women will develop breast cancer during her lifetime (Finnish Cancer Registry, 2000). Breast cancer has many known risk factors. The most important are young age of menarche, late menopause, late age at first birth, nulliparity, high social class, obesity, benign proliferative breast disease, and family history of breast cancer (Dupont and Page, 1987; Madigan et al., 1995). The diagnosis is made by palpation, mammography, or fine needle or thick needle biopsy. Most often (70-80%) women find the palpable lump themselves. Other symptoms are pain, skin changes, discharge from the nipple, or general symptoms. However, often women are symptomless (Dixon and Mansel, 1994). Population programmes to screen for specific age-groups are already carried out in many countries with public health care systems. In Finland, in general, women aged 50 to 59 are invited every two years to mammography screening; screening is considered to reduce the risk of dying from breast cancer by about 25% (Kerlikowske et al., 1995; Hakama et al., 1997). This is especially clear with post-menopausal women, but the effect for pre-menopausal women is not that clear (Kerlikowske et al., 1995).

Treatment of breast cancer is based on stage and some other prognostic factors and on the age and general condition of the patient. Previously, total mastectomy and evacuation of the axilla were the basic treatment, but nowadays breast-conserving surgery is quite common and is done for 30 to 60% of the breast cancers in Finland depending on hospital (Asko-Seljavaara and von Smitten, 1999). Besides surgery, other important treatment options are radiation, chemotherapy, or hormone treatment (Harris et al., 1996).

The prognosis for breast cancer has greatly improved during recent decades. The five-year relative survival rate (RSR), based on Finnish Cancer Registry data, is nowadays about 80% (Dickman et al., 1999). Still, prognosis varies widely between different stages. From 1985 to 1994 in Finland, the five-year RSR was 93%, 69%, and 22% for the patients with localised disease, regional metastases, and distant metastases, respectively (Dickman et al., 1999).

Hereditary predisposition to breast cancer

One of the strongest risk factors for breast cancer is family history (Colditz et al., 1993; Madigan et al., 1995), with epidemiological studies showing an increase in breast cancer incidence in relatives of breast cancer patients. Risk of breast cancer is about two-fold in first-degree female relatives above that for women with no affected relative (Sattin et al., 1985; Claus et al., 1990; Houlston et al., 1992; Tulinius et al., 1992; Pharoah et al., 1997; Ziogas et al., 2000), and it is even more pronounced if the breast cancer of the relative has been diagnosed at a young age (Claus et al., 1990; Houlston et al., 1992; Tulinius et al., 1992; Pharoah et al., 1997), or if the relative has bilateral disease (Houlston et al., 1992; Tulinius et al., 1992). With two affected first-degree relatives, the risk has been documented to be from 2.5- to 13.6-fold (Pharoah et al., 1997).

Breast cancer families form a large heterogeneous group. About 30% of breast cancer patients report at least one relative affected by the disease (Lynch et al., 1988; Thompson, 1994). Recently, a large twin study (Lichtenstein et al., 2000) combining the information of twin registries from Denmark, Finland, and Sweden showed that hereditable factors played a role in about 30% of the breast cancers. Earlier estimations have been lower, and genetic factors have been documented to be involved in about 5 to 19% (Colditz et al., 1993; Slattery and Kerber, 1993; Madigan et al., 1995).

Roughly, the families can be classified into three somewhat overlapping groups: high-risk families, moderate-risk families, and families with sporadic clustering of breast cancer. High-risk families are those in which the segregation of a susceptibility gene has clearly manifested itself in multiple cases of breast cancer in close relatives over several generations. These families are often characterised by an early age at diagnosis of cancer, or by the presence of bilateral or multiple primary breast cancers (Eeles, 1999). Genetic models have provided evidence for a rare autosomal dominant high penetrance gene(s) for susceptibility to breast cancer, with a frequency of 0.0006 to 0.0033 in the general population (Newman et al., 1988; Claus et al., 1991). Elevated risk of ovarian cancer has also been found among breast cancer patients' relatives (Schildkraut et al., 1989; Tulinius et al., 1992), and a breast/ovarian cancer susceptibility gene(s) is estimated to account for about 7% of breast cancer cases in the general population (Claus et al., 1996). Two major

dominantly inherited genes predisposing to breast and ovarian cancer have been identified, BRCA1 (Miki et al., 1994) and BRCA2 (Wooster et al., 1995). Mutations in these genes account for a large proportion of the high-risk families, but it is suggested that other susceptibility genes exist as well (Schubert et al., 1997; Serova et al., 1997; Peto et al., 1999; Kainu et al., 2000); also autosomal recessive inheritance, or a number of common low-penetrance genes, with additive effects, have been suggested to account for the residual non-BRCA1/2 familial aggregation of breast cancer (Antoniou et al., 2001; Cui et al., 2001).

Breast cancer susceptibility may also be associated with rare germline mutations that cause other cancer predisposition syndromes, and these explain a small proportion (<1%) of hereditary breast cancers. Examples of these are mutations in the p53 gene causing the Li-Fraumeni syndrome (Sidransky et al., 1992), PTEN and the Cowden syndrome (Li et al., 1997), and STK11 (or LKB1) and the Peutz-Jeghers syndrome (Hemminki, 1999). Mutations in CHK2, p16, the androgen receptor gene, and in the oestrogen receptor gene are suggested to predispose to breast cancer, as well (Welcsh et al., 1998; Bell et al., 1999; Borg et al., 2000).

Families with a moderate risk of breast cancer are characterised by a less striking family history, and the increased risk may be due to low penetrance genes, multiple genetic factors, or shared environmental factors. Some families may be affected by mixtures of these factors or an interaction of genes and environment. In the third group of families, there is sporadic clustering of cancer purely due to chance, because breast cancer is a very common disease. And opposed to this are those patients with mutations in high-risk genes without any family history at all (Figure 1).

Low-penetrance genes for breast cancer, with disease-associated variant alleles, may be relatively common at the population level and as such may be associated with a much higher attributable risk in a population (Rebbeck, 1999; Weber and Nathanson, 2000). Heterozygous mutations in the ATM gene (Olsen et al., 2001) causing ataxia-telangiectasia, an autosomal recessive genetic neurological disorder, and mutations in a number of candidate genes that play a role in the metabolism of environmental carcinogens have been suggested to cause a slightly increased risk for disease (Rebbeck, 1999; Weber and Nathanson, 2000).

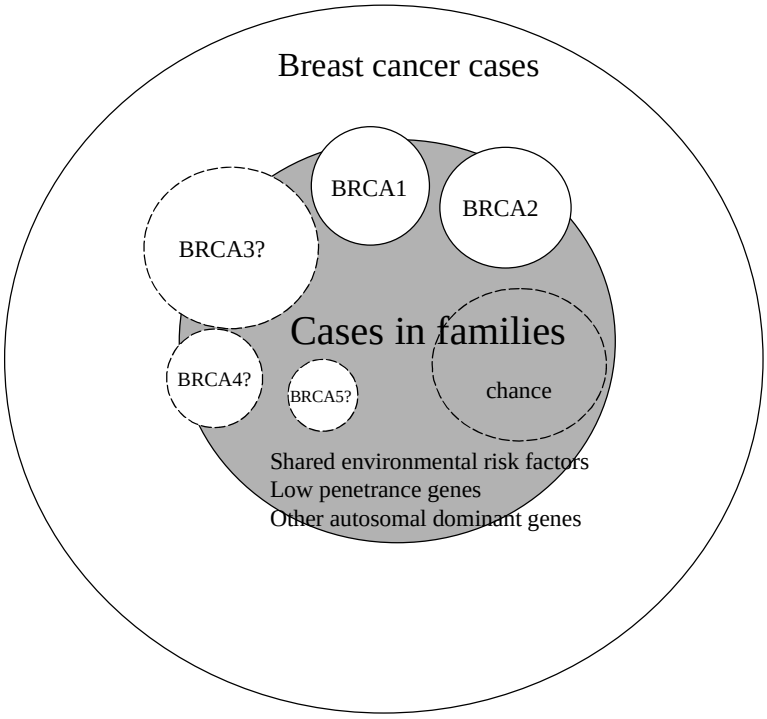

Figure 1. About 30% of breast cancer patients have some family history of breast cancer. Some are true hereditary cases (due to different genes), with others due to shared environmental factors or occurring by chance.

Multi-step development of cancer

Carcinogenesis is a multi-step process, with several genetic changes in tumour suppressor genes or oncogenes needed for malignant transformation. Tumour suppressor genes can be classified as the so-called "gatekeepers" controlling the cell cycle or "caretakers" responsible for genomic integrity by DNA repair (Kinzler and Vogelstein, 1997). Germ-line mutations in tumour-suppressor genes act in the cells in a recessive manner, which is explained in Knudson's two-hit model (Knudson, 1971): in hereditary cancer predisposition, the first mutated allele is inherited and exists in every cell of the body. To develop cancer, somatic changes also have to occur in the cells, and this "second hit" causes the normal allele to be lost. However, because only one allele has to be inherited, in human beings the mode of inheritance is thus dominant. In sporadic tumours, both "hits" occur in the somatic cells.

Mutations in tumour-suppressor genes have been documented in many cancers including breast cancer. The p53 gene is a major gatekeeper gene controlling the cell cycle in response to DNA damage either by arresting it, allowing apoptosis, or stimulating DNA repair (Levine, 1997). DNA-repair genes act like caretakers and recognise and repair DNA damage. Germ-line mutations in DNA-mismatch repair genes predispose, for example, to HNPCC syndrome (Aaltonen et al., 1994). BRCA1 and BRCA2 have been suggested to play a role in both gatekeeping and caretaking (Kinzler and Vogelstein, 1997).

Oncogenes promote cell proliferation. At the cellular level, the oncogenes act in a dominant manner, meaning that one allele alone can promote cell proliferation. Multiple endocrine neoplasia 2 (MEN2) and familial medullary thyroid carcinoma are known to be caused by mutations in an oncogene, RET2 (Goodfellow and Wells, 1995). Genomic instability, as a consequence of mutations in caretaking genes or oncogenes, may lead to accumulation of defects at many other sites, or to mutations in gatekeeper genes directly, leading to an uncontrolled cell-cycle and to tumourigenesis (Kinzler and Vogelstein, 1997).

Major breast cancer predisposing genes BRCA1 and BRCA2

The BRCA1 gene is located in 17q21 (Hall et al., 1990). It is a large gene with 24 exons, of which 22 encode a protein of 1863 amino acids (Miki et al., 1994). The BRCA 2 gene is located in 13q12-13 (Wooster et al., 1994). It is even larger than BRCA1, including 27 exons, of which 26 encode a protein with 3418 amino acids (Tavtigian et al., 1996). Nearly 900 mutations and sequence variants have been reported in the BRCA1 gene and the same number in BRCA2. An online summary of these mutations is available from the Breast Cancer Information Core (BIC) (on the Internet: www.nchgr.nih.gov/Intramural_research/Lab_transfer/Bic/). All classes of mutations are represented: missense mutations, nonsense mutations, deletions, and insertions. Mutations occur throughout the coding sequence, and in a few cases those exist in regulatory sequences. Most disease-associated mutations result in premature termination codons (Shattuck-Eidens et al., 1995; Tavtigian et al., 1996). Usually these mutations are due to small alterations in the gene,

although large deletions have been identified. Most mutations are unique to one or a few families, but prevalent founder mutations exist as well (Breast Cancer Information Core, 2001).

Loss of heterozygosity (LOH) and deletion of the wild type allele of BRCA1 and BRCA2 is seen in tumours of the mutation carriers; thus, in accordance with Knudson's two-hit hypothesis they seem to be tumour-suppressor genes. The protein structures and interactions with other proteins have been investigated to find clues to their functions. The proteins seem to be multifunctional (Zheng et al., 2000 and reviewed in Welcsh and King, 2001; Venkitaraman, 2001). Both genes seem to be essential for the maintenance of chromosome stability. BRCA1- and BRCA2-proteins interact with other proteins involved in DNA repair, for example with Rad51, forming complexes which seem to be essential for homologous recombination and double-stranded break repair (Sharan et al., 1997; Chen et al., 1998). Experimental data suggest that these genes are regulators of transcription (Monteiro et al., 1996; Milner et al., 1997). Furthermore, they seem to be required for a normal proliferation burst in early embryogenesis (Gowen et al., 1996; Liu et al., 1996; Sharan et al., 1997), and they are upregulated along with proliferation of the breast epithelial cells during puberty, pregnancy, and lactation (Rajan et al., 1997).

Detection of BRCA1 and BRCA2 mutations

After the BRCA1 and BRCA2 genes were localised but not yet identified, molecular genetic studies were based on linkage analysis. This means that in large families, with several affected members, genetic studies could be carried out by studying the inheritance of the markers in the chromosomal region linked to the disease (the putative gene).

Once the genes were identified, a direct test on a single individual could be done. However, both genes are very large with many mutations scattered throughout the large coding regions of the genes. Furthermore, most mutations appear uniquely in single families (Breast Cancer Information Core, 2001). A variety of methods to scan for unknown mutations are available. Scanning can be done by sequencing the whole gene, but it is laborious and expensive and done routinely in only a few laboratories. Other more common methods, such as single-stranded conformation polymorphism (SSCP),

heteroduplex analysis, or denaturing gradient gel electrophoresis (DGGE), are based on altered migration of a mutation-containing sequence in electrophoresis. Mutation can be detected also by cleavage of a mismatch between normal and mutant DNA, or - if the mutation causes a stop codon in the gene – then by detection of a truncated protein product (PTT). All these techniques have their strengths and weaknesses, and mutation detection may not always be successful even if complete coding sequence and splice sites of both large genes are analysed. Testing with these methods is also laborious, time-consuming, and expensive. In some populations, recurrent founder mutations account for a large proportion of the families. Screening for only the founder mutations is less laborious but also means that a higher percentage of the families with mutations are not detected (Cotton et al., 1998).

After the mutation that is segregating in a family has been identified by sequencing the region, testing of the known mutation in family members is possible. All of the methods are based on PCR amplification of the region of the gene containing the mutation. The mutation identified can be studied, for example, by detecting altered mobility of the sequence in electrophoresis or by detecting alteration at the site of the restriction enzyme (restriction fragment length polymorphism analysis, RFLP) or the allele-specific oligonucleotide (ASO) assay method, in which a radioactively labelled oligonucleotide probe will bind only to the exact complementary sequence, either normal or mutated. Allele-specific amplification means that amplification occurs only if the primer perfectly matches with the sequence studied. In minisequencing, the primer can be extended only when the base complementary to the sequence is provided in the reaction mix (Cotton et al., 1998).

Prevalence

BRCA1 and BRCA2 were originally thought to account for about 80% of all hereditary susceptibility to breast cancers. However, recent studies suggest that they account for the susceptibility in only about 30% of all families with a strong history of breast cancer in most populations, and this proportion seems to vary according to population (Szabo and King, 1997). Some populations have very prevalent founder mutations. However, studies in which definitions of family history differ are not easy to compare. Ford et al. (1998)

found that among families with at least four cases of breast cancer diagnosed before age 60, the disease was linked to BRCA1 in more than half the families and to BRCA2 in more than a third of the families. The majority of families (80%) with ovarian cancer were linked to BRCA1, while families with male cases of breast cancer were mainly linked to BRCA2 (77%) (Figure 2). In families with neither ovarian cancers nor male breast cancer cases, lower proportions of the mutations are suggested. In the same study, only 32% of families with four or five female breast cancers, all diagnosed before age 60, were linked to BRCA1 and only 9% to BRCA2 (Ford et al., 1998). Of 23 families without ovarian or male breast cancer cases, Serova et al. (1997) found mutations in eight (34%), with three or more close relatives affected with breast cancers, all of which were under 60, and with at least one affected before age 45. In Sweden, of the 106 families with at least three affected first-degree relatives, (one below age 50), or with two affected (one below age 40), or one affected (before age 30), 24 (23%) were BRCA1-positive; of these 106, 12 (11%) families were BRCA2-positive (Håkansson et al., 1997).

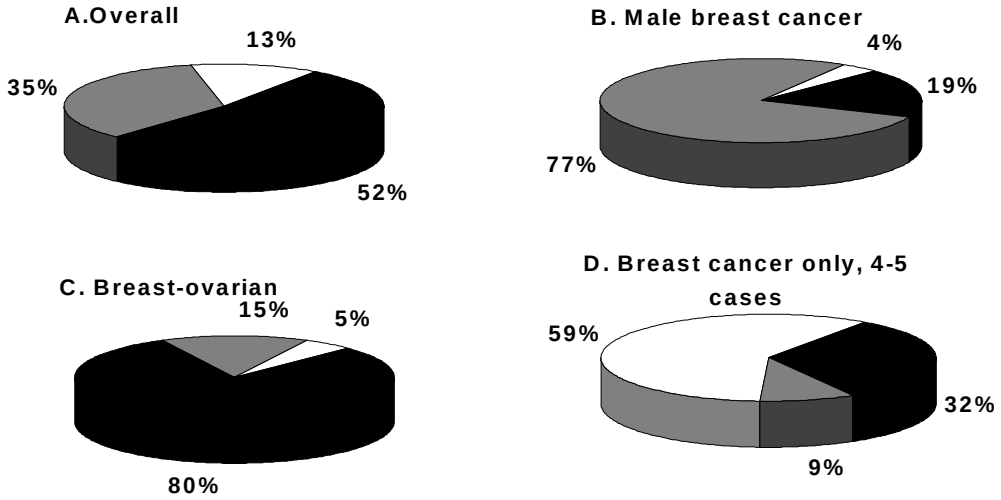

Figure 2. BRCA1 (black) and BRCA2 (gray) linked breast cancer families and mutation-negative (white) families with at least 4 breast cancer cases diagnosed at age <60. (Data from Ford et al. Am J Hum Genet 62: 676-689, 1998) A. Overall, B. In families including male breast cancer case(s), C. In families including ovarian cancer case(s), D. In families without ovarian or male breast cancer cases and only 4 to 5 breast cancer cases

Mutations in the BRCA1 and BRCA2 genes occur throughout these large genes, making the screening for mutations challenging. Thus, studies have rarely reported estimates of mutation frequencies among unselected breast cancer patients. Usually only a few hundred

patients have been analysed in each study, with mutation frequencies ranging from 0.4 to 12% for BRCA1 and from 1.3 to 10.7% for BRCA2 (Table 1).

Table 1. Prevalence of BRCA1 and BRCA2 in population-/hospital-based series of breast cancer patients

					age						
Study	Population	No. tested	Method	Age-group	<35	<40	<45	<50	<55	<75	all
BRCA1											
Krainer et al. 1997	USA (white)	73	P	<32	12% a						
Langston et al. 1996	USA (white)	80	W	<35	7.5%						
Malone et al. 1998	USA (white)	193	W	<35	6.2%						
Southey et al. 1999	Australian	91	W	<40	-	3.8% e					
Hopper et al. 1999	Australian	388	P	<40	-	2.3%					
Loman et al. 2001	Swedish	234	W	<41	-	6.8% d					
Peto et al. 1999	UK	617	W	<45	3.5% b	-	2.6%	3.1% e		1.3 ce	
Anglian BC SG 2000*	UK	1435	W	<55	4.1%	-	1.3%	-	0.7%	-	
Newman et al. 1998	USA	211	W	All ages	-	-	-	-	-	1.4%	
Papelard et al. 2000	Dutch	642	P	All ages	-	7.1%	-	4.8%	-	-	1.6%
Syrjäkoski et al. 2000	**Finnish**	**1035**	**F**	**All ages**	**3.6%**	**2.8%**	**1.4%**	**1.0%**	**0.6%**	**0.4%**	**0.4%**
Van der Looij et al. 2001	Hungarian	500	F	All ages	-	-	-	5.5%	-	-	3.4%
BRCA2											
Krainer et al. 1997	USA (white)	73	P	<32	2.7 a						
Hopper et al. 1999	Australian	388	P	<40	-	2.3%					
Loman et al. 2001	Swedish	234	W	<41	-	2.1%d					
Peto et al. 1999	UK	617	W	<45	2.4% b	-	2.3%	3.0% e		1.5 ce	
Anglian BC SG 2000*	UK	1435	W	<55	8.3%	-	2.0%	-	1.3%	-	
Syrjäkoski et al. 2000	**Finnish**	**1035**	**F**	**All ages**	**10.7%**	**7.0%**	**3.6%**	**2.1%**	**1.7%**	**1.6%**	**1.4%**
Van der Looij et al. 2001	Hungarian	500	F	All ages	-	-	-	0.6%	-	-	0.2%

a= <32y, b=<36, c=<70y, d=<41, e=estimation, P=partial screen, F=founder mutations, W=whole coding region
Some of the proportions by Syrjäkoski et al. are unpublished.
*Anglian Breast Cancer Study Group

Individual, highly recurrent founder mutations have been reported in Ashkenazi Jews (Hartge et al., 1999). Up to 2.3 to 2.6% of all Ashkenazi Jews carry one of the three founder mutations: BRCA1 185delAG (0.7-1.1%), BRCA2 6174delT (1.1-1.4%), or BRCA1 5382insC (0.1- 0.4%) (Tonin et al., 1996; Hartge et al., 1999), and about 12% of unselected breast cancer patients carry one of these three founder mutations (Warner et al., 1999). The mutation 999del5 is found in 8.5% of Icelandic breast cancer patients, in 27% of female breast cancer patients diagnosed at age 30 to 39 years, and in 7.9% of ovarian cancer patients (Johannesdottir et al., 1996).

BRCA1 and BRCA2 mutations in Finland

In Finland, BRCA1 and BRCA2 mutations have been detected in a low proportion of breast cancer families. Among families with three affected first- or second-degree relatives without age restrictions, the proportions were 10% and 11% for BRCA1 and BRCA2, respectively (Vehmanen et al., 1997 a; Vehmanen et al., 1997 b). The highest proportions have been found in families which also include ovarian or early onset breast cancer (Table 3) (Vahteristo et al., 2001). Among unselected breast cancer patients, BRCA1 and BRCA2 mutations were found in 1.8% (Table 1) (Syrjäkoski et al., 2000). Among breast cancer cases, BRCA2 seems to be more prevalent in Finland (1.4% vs. 0.4% BRCA1), whereas BRCA1 founder mutations were detected in 4.7% and BRCA2 in 0.9% of 233 unselected ovarian cancer cases (Sarantaus et al., 2001).

Table 2. Number of mutation-positive families by family background (modified from Vahteristo et al.,2001)

	All families	BRCA1	BRCA2	Mutation detected %
3 affected	74	6	2	10.8
only breast, none under 40	47	0	1	2.1
only breast, some under 40	15	1	0	6.7
breast and ovarian, none under 40	9	3	0	33.3
breast and ovarian, some under 40	3	2	1	100
4 affected	35	5	3	22.9
only breast, none under 40	15	0	0	0
only breast, some under 40	7	1	0	14.3
breast and ovarian, none under 40	11	3	1	36.4
breast and ovarian, some under 40	3	1	2	100
>5 affected	39	5	8	33.3
only breast, none under 40	6	0	0	0
only breast, some under 40	10	0	2	20.0
breast and ovarian, none under 40	9	1	0	11.1
breast and ovarian, some under 40	14	4	6	71.4
total	148	16	13	19.6
only breast, none under 40	68	0	1	1.5
only breast, some under 40	32	2	2	12.5
breast and ovarian, none under 40	28	7	1	28.6
breast and ovarian, some under 40	20	7	9	80.0

At the time of Study IV, fifteen distinct BRCA1 mutations and eight BRCA2 mutations were detected. Seven of the BRCA1 and seven of the BRCA2 were recurrent (Table 2) (Vehmanen et al., 1997 a; Vehmanen et al., 1997 b; Huusko et al., 1998; Tapper et al.,

1998; Sarantaus et al., 2000; Vahteristo et al., 2001). Some of these mutations are unique to Finland, while others are found also elsewhere.

Table 3. BRCA1 and BRCA2 mutations in Finland

Gene and mutation	U/R	Gene and mutation	U/R
BRCA1		BRCA2	
1047 C->T	U	999del5	R
1806C->T	R	4081insA	R
1924delA	U	5797G->T	R
2592insA	U	6495/6496G→C, delCA	U
2804delAA	R	6503delTT	R
3264delT	U	7708C→T	R
3604delA	R	8555T→G	R
3745delT	R	9346nt-2A→G	R
3904C→A	U		
4154delA	U		
4216nt-2A→G	R		
4446C→T	R		
5145del11	U		
5370C→T	R		
5382insC	U		

U=Unique mutation detected in a single family
R=Recurrent mutation detected in multiple families

Of the recurrent founder mutations, 11 have been discovered to account for a large majority of the Finnish BRCA1/2 families found in screening of both of the genes in their entirety (Vehmanen et al., 1997 a; Vehmanen et al., 1997 b; Sarantaus et al., 2000). Furthermore, the linkage analysis in 24 of Finnish large mutation-negative families suggested possible linkage to either BRCA1 or BRCA2 in only four of the families (Tommi Kainu, personal communication). The majority of Finnish founder mutations thus may already have been identified. Haplotype analysis has indicated that the families carrying the founder mutations have common ancestors. The spread of the different mutations in the Finnish population started at different times from 23 to 36 generations ago to 7 to 9 generations ago, and it seems that the ancestors of the families with the same founder mutations cluster in well-defined geographical regions of the country. Thus, even today, in some parts of the country, the mutation spectrum is very narrow, while the widest spectrum of mutations has been detected in the Helsinki region (Sarantaus et al., 2000).

Risk of cancer

The clinical expression of BRCA1 and BRCA2 appears to vary from family to family. These differences may be due to different mutant alleles, to the influence of allelic variation in other genes on the same phenotype, to random accumulation of mutations in other critical genes, or to environmental factors such as modification of cancer risk by pregnancy or by use of exogenous hormones. Reports show wide variation in the risk that carriers of these mutations will develop breast cancer depending on population or on mutation position studied (Table 4) (Ford et al., 1994; Gayther et al., 1997; Struewing et al., 1997; Ford et al., 1998; Thorlacius et al., 1998; Thompson et al., 2001).

Studies based on families with multiple cases have found a very high lifetime risk of breast cancer and varying risks for ovarian cancer. The cumulative breast cancer risk for BRCA1 gene carriers has been estimated to be 85 to 87% and the ovarian cancer risk 44 to 63% by the age of 70 (Ford et al., 1994; Easton et al., 1995). BRCA2 mutations seem to predispose to a risk of breast cancer equally high as that of BRCA1 (77-84% by age 70) but to a lower risk of ovarian cancer (16-27%) (Ford et al., 1998; Breast Cancer Linkage Consortium, 1999). A few studies have shown that BRCA2 carriers develop breast cancer at older ages than do BRCA1 carriers. The risk of subsequent ovarian or breast cancer has also been reported to be very high in both groups. Over half the patients in both groups were affected with contralateral breast cancer by age 70, and 44% of those with BRCA1 mutations and 16% with BRCA2 mutations developed subsequent ovarian cancer by age 70 (Ford et al., 1994; Breast Cancer Linkage Consortium, 1999).

Risk estimates in population-based studies have been lower (Levy-Lahad et al., 1997; Struewing et al., 1997; Thorlacius et al., 1998; Warner et al., 1999; Antoniou et al., 2000; Risch et al., 2001; Satagopan et al., 2001). In the Ashkenazi population, a 28 to 57% cumulative risk of breast cancer (Struewing et al., 1997; Warner et al., 1999) and a 16% risk of ovarian cancer (Struewing et al., 1997) by the age of 70 has been documented for their three BRCA1 and BRCA2 founder mutations. The risk of breast cancer associated with the Icelandic BRCA2 founder mutation 999del5 has been estimated to be 37% by the age of 70 (Thorlacius et al., 1998). In England and Australia, with their more mixed populations and wider mutation spectra, low risks have been observed as well (Hopper et al., 1999; Peto et al., 1999). Peto et al. estimated also the standardized incidence ratio for

breast cancer in mothers and sisters of the BRCA1 and BRCA2 mutation carriers to be 3.65 (five cases observed, 1.37 expected), and Hopper et al. estimated that the risk among carriers is on average nine times as high as that of the general population.

*Table 4. Breast and ovarian cancer risk by age 70 for BRCA1 and BRCA2 carriers in families with multiple cases (M) affected and in families from population-based series (P) of breast cancer patients**

	Breast cancer		Ovarian cancer	
	Risk	Study population	Risk	Study population
BRCA1	87% M	Ford et al.1994	44% M	Ford et al.1994
	60% P (Askhenazi Jewish)	Warner et al. 1999	66% P (ovca)	Antoniou et al. 2000
	47% P (cases <55y)	Anglian BC SG 2000**	36% P (cases <55y)	Anglian BC SG 2000**
	45% P (ovca)	Antoniou et al. 2000		
	46% P (Askhenazi Jewish)	Satagopan et al. 2001		
BRCA2	84% M	Ford et al.1998	27% M	Ford et al.1998
	37% P (Icelandic)	Thorlacius et al. 1998	16% M	BCLC 1999
	77% M	BCLC 1999	10% P (cases <55y)	Anglian BC SG 2000**
	28% P (Askhenazi Jewish)	Warner et al. 1999		
	56% P (cases <55y)	Anglian BC SG 2000**		
	26% P (Askhenazi Jewish)	Satagopan et al. 2001		
BRCA1/2	57% P (Askhenazi Jewish)	Struewing et al. 1997	16% P (Askhenazi Jewish)	Struewing et al. 1997
	40% P cases <40y)	Hopper et al. 1999	16% P (cases <55y)	Anglian BC SG 2000**
	54% P (cases <55y)	Anglian BC SG 2000**		

* In one study, families came from population-based series of ovarian cancer patients (ovca), and some of the studies had age restrictions
**Anglian Breast Cancer Study Group

Risk of cancer may also associate with the site of the mutation within the gene, and it is documented that in the BRCA1-positive families, the proportion of ovarian to breast cancer is higher in mutations situated at the 5' end of the gene than at the 3' end (Gayther et al., 1995; Sarantaus et al., 2000), and that risk of breast cancer increases towards the 3' end (Risch et al., 2001). It has been suggested as well that germline mutations in the "ovarian cancer cluster region" (OCCR), in the middle part of the BRCA2 gene confer a greater risk of ovarian cancer and lower risk of breast cancer than do the mutations in the other parts of the gene (Gayther et al., 1997; Thompson et al., 2001). The OCCR region is associated with about 20% and the outer regions about 11% risk of ovarian cancer by age 70, while the risk of breast cancer is lower, about 33% in the OCCR region and 46% outside the region by age of 70 years (Thompson et al., 2001).

A few studies have discovered among BRCA1 and BRCA2 families increased risks of cancers other than breast or ovarian (Ford et al., 1994; Struewing et al., 1997; Breast Cancer Linkage Consortium, 1999; Johannsson et al., 1999). However, most of the findings of increased risks have been detected only once, without confirmation by later studies.

Some of the largest studies report an elevated risk of prostate cancer in both groups of families (Ford et al., 1994; Struewing et al., 1997; Breast Cancer Linkage Consortium, 1999; Warner et al., 1999), while Johannsson et al. (1999) found it to be higher only in BRCA2-associated families. Thorlacius et al. (1997) found an increased incidence of prostate cancer (RR=3.46, 95% CI 1.83-5.81) among relatives of carriers of BRCA2 999del5 mutations, as well (Thorlacius et al., 1997). Recently, in the BCLC study (Thompson et al., 2001), the risk of prostate cancer by age 80 in BRCA2 carriers was found to be 33.6% for non-OCCR mutations and 19.2% for OCCR mutations. However, studies of the prevalence of BRCA1 and BRCA2 mutations among prostate cancer patients suggest only a minor role for these mutations in prostate cancer in general (Langston et al., 1996; Sinclair et al., 2000).

Ford et al. (1994) suggest that the risk of colon cancer is elevated among BRCA1 families (RR=4.11, 95% CI 2.36-7.15). In BRCA1-associated families, Johannsson et al. (1999) reported increased incidences only of stomach cancer (standardized mortality ratio (SMR)=5.86, 95% CI 1.60-15.01) among women and of invasive squamous cell cancer of the skin among men (SMR=6.02, 95% CI 1.96-14.05). In BRCA2 families Johannsson et al. suggested that besides prostate cancer, the risk of invasive cervical cancer may be increased (SMR=4.21, 95% CI 1.15-10.79), and The Breast Cancer Linkage consortium (1999) found increased risks of pancreatic (RR=3.51, 95% CI 1.87-6.58), gallbladder and bile duct (RR=4.97, 95% CI 1.50-16.52), and stomach cancers (RR=2.59, 95% CI 1.46-4.61) and malignant melanoma (RR= 2.58, 95% CI 1.28-5.17). Increased incidence of pancreatic cancer was also seen among relatives of BRCA2 999del5 carriers in Iceland (RR=2.18, 95% CI 1.27-3.48) (Thorlacius et al., 1997).

Biological features of the tumours

Several studies have compared the pathology of breast cancers in BRCA1 carriers and in sporadic controls. BRCA1-associated tumours are aggressive and show features associated with poor prognosis, e.g., high tumour grade, oestrogen receptor negativity, and over-expression of p53 (Noguchi et al., 1999; Foulkes et al., 2000 and reviewed by Phillips et al., 1999). Some studies have shown features that indicate a good prognosis, such as a higher proportion of medullary and atypical medullary carcinomas (Malone et al., 1998; Phillips et al., 1999) and tumours with low expression of ERBB2 (Noguchi et al., 1999; Phillips et al., 1999). Besides the higher proportion of medullary histology, a higher frequency of ductal carcinoma has also been reported (Eisinger et al., 1996; Johannsson et al., 1997).

Among BRCA2-associated tumours, a slight increase has been observed in the incidence of lobular or tubulolobular carcinomas (Marcus et al., 1996; Armes et al., 1998). However, results are inconsistent, and in most case no significant difference has been found between BRCA2-associated and the sporadic cancers (Noguchi et al., 1999; Phillips et al., 1999; Vahteristo et al., 2001).

Recently, a cDNA microarray technique has been used to identify the gene expression profile of several thousands of genes in breast tumours. This approach is useful in sub-classification of breast cancer (Perou et al., 2000). Gene-expression profiles in hereditary breast cancer have also undergone study, and BRCA1- and BRCA2-associated tumours could be distinguished by their gene expression profiles from each other as well as from sporadic tumours (Hedenfalk et al., 2001).

Prognosis of breast cancer patients

Despite several reports of biological indicators of poor outcome among BRCA1 patients, many of the studies have shown no difference in their prognosis compared to that of sporadic cases (Hamann and Sinn, 2000 and reviewed by Chappuis et al., 1999). A few recent studies, however, have suggested a worse outcome (Foulkes et al., 1997; Ansquer et al., 1998; Johannsson et al., 1998; Robson et al., 1999; Foulkes et al., 2000; Stoppa-

Lyonnet et al., 2000). Differences between studies may be due to methodological differences or be dependent on the study or the control population. A few studies are based on the findings of founder mutations, and in every case the number of patients (9-48) has been small.

Only a few studies have reported on survival in patients with BRCA2-associated breast cancer (Verhoog et al., 2000). Loman et al. (2000) documented BRCA2-asssociated cases with a poorer survival than their sporadic controls. However, after adjustment for stage, the breast cancer-specific survival was no longer significantly worse (Loman et al., 2000). Others have found no significant differences (Gaffney et al., 1998; Lee et al., 1999; Verhoog et al., 1999). However, the number of patients in all of these studies, as well, has been small (20-54 patients).

Non-BRCA1/2 families

It has been clear for some time that in many families hereditary susceptibility is not linked to the BRCA1 or BRCA2 gene (Schubert et al., 1997; Serova et al., 1997; Ford et al., 1998). Ford et al. (1998) found that among families with at least four cases of breast cancer diagnosed before age 60, the proportion of families without BRCA1 or BRCA2 linkage was 13%. Furthermore, when families with precisely four to five affected family members were studied, among whom there were neither affected males nor patients with ovarian cancer, the families not linked to BRCA1 or BRCA2 formed the largest proportion (59%) (Figure 2). Serova et al. (1997) achieved a similar result when they studied the prevalence of the mutations in site-specific breast cancer families and found that 66% of the families were mutation-negative. In Finland, as well, the proportion of BRCA1/2 mutation-negative families is high among site-specific breast cancer families (Table 2). Peto et al. (1999) found similar results in their population-based series of breast cancer patients in England, and concluded that only about 16% of overall familial risk appears to be due to BRCA1 or BRCA2.

Lakhani et al. (2000) found non-BRCA1/2 cancers to be of lower grade ($p=0.001$), to show less pleomorphism ($p= 0.0002$), and to have a lower mitotic count ($p=0.003$) than sporadic cancers. No other features significantly differed. Up to now, no other studies

have evaluated non-BRCA1/2 families, and to our knowledge, neither risk of cancer nor patient survival has been previously studied among non-BRCA1/2 families.

Most of the familial risk may be due to other genes, which may have lower penetrance than BRCA1 or BRCA2 but which may be more prevalent in the population (Peto et al., 1999). Other susceptibility genes are sought (Kainu et al., 2000), and autosomal recessive inheritance or a number of common low-penetrance genes with additive effects has also been suggested to account for some of the non-BRCA1/2 families (Antoniou et al., 2001; Cui et al., 2001).

Identifying high risk families

In general, selection of families for genetic testing is based on the family history as recalled by the patient. Cloning of BRCA1 and BRCA2 genes has made predictive diagnostic tests of cancer predisposition possible in some families. However, in many families no causative gene or mutation can be found and risk assessment is based solely on family history.

Factors that speak for hereditary background are: multiple cases in the family, young age of onset, multiple tumours in a single patient. Occurrence of both breast and ovarian cancer in the family is strongly associated with hereditary predisposition for cancer, especially for the risk of carrying BRCA1 or BRCA2 mutations. Today there exist many models or computer software to test the probability for BRCA1 or BRCA2 mutations (Couch et al., 1997; Shattuck-Eidens et al., 1997; Chan-Claude et al., 1998; Parmigiani et al., 1998; Gilpin et al., 2000; Vahteristo et al., 2001). The strongest predictors for BRCA1 and BRCA2 mutations are early age of breast cancer onset and number of ovarian cancer cases in the family (Vahteristo et al., 2001). Simple family history criteria of the strongest predictors (under age 40 breast cancer onset and presence of ovarian cancer) for a mutation may also serve as a rough estimation of a high likehood of carrying a mutation (Vahteristo et al., 2001).

Identifying families and the individuals at high risk of breast cancer, based on information about the family is not, however, simple, especially when there is no strong indication of

BRCA1 or BRCA2 involvement. Breast cancer is a common disease, and there may be clustering purely by chance or due to accumulation of environmental risk factors. A few methods have been developed to distinguish the true high-risk individuals. The model developed by Claus et al. (Claus et al., 1994) gives estimations of the cumulative probability over age that an unaffected woman will be diagnosed with breast cancer based on her family history. It incorporates information about first- and second-degree relatives with breast cancer and their age at diagnosis. Another model, by Gail et al. (1989), includes, besides the number of first-degree relatives, risk factors other than family history. A software program is available from the National Cancer Institute at http://brca.nci.nih.gov/brc/. A few other models have been developed for genetic counselling purposes (Amstrong et al., 2000). If unaffected relatives are not taken into account, over-estimation of the risks may result, if the family is large (Schmidt et al., 1998).

Accuracy of family history recalled by patients

A few study groups have assessed the accuracy of reported family history of breast cancer. Accuracy is considered to be high especially regarding first-degree relatives, although less accurate data are available about breast cancer in the second-degree relatives (Love et al., 1985; Theis et al., 1994; Douglas et al., 1999) or regarding some other cancer sites (Love et al., 1985; Koch et al., 1989; Theis et al., 1994; Kerber and Slattery, 1997; Gladstone et al., 1998; Douglas et al., 1999; Sijmons et al., 2000). Interestingly, in a large U.S. study, the Breast Cancer Detection Demonstration Project, in which 1 362 breast cancer patients were detected and interviewed for family history and other risk factors, only a small proportion of subjects were able to recall information on the cancer status of their grandmothers (21% for the question regarding paternal grandmother and 13% for the maternal grandmother) (Brinton et al., 1982). Furthermore, a false family history is a documented phenomenon with serious implications for risk assessment and clinical management (Kerr et al., 1998). Altogether, for clinical family counselling, a correct family history is critical in risk estimation as well as in treatment and follow-up decisions.

Surveillance and management

Most centres in Europe recommend surveillance of the breasts if the lifetime risk exceeds 15 to 20% (Vasen et al., 1998). This includes monthly self-breast examinations, examination by specialist every six months and annual mammography, starting from an age between 25 and 35. Sonography is performed only in patients with suspicious breast lesions; MRI, which has the high sensitivity needed in the screening of high-risk women, is thus far limited to research. Surveillance of the ovaries is recommended for BRCA1- and BRCA2-carriers or for families with ovarian cancer cases; it includes a gynaecological examination, sonography, and measurement of CA-125 at yearly intervals starting from ages 30 to 35. The efficacy of such types of surveillance is not yet known.

Chemoprevention is under investigation and not routinely used. Few studies have evaluated the prevention of breast cancer by Tamoxifen: In America, the National Surgical Adjuvant Breast and Bowel Study showed a significant reduction (49%) in risk of breast cancer among high-risk women (Fisher et al., 1998), but two other studies, one with hysterectomised women from Italy and one with English women having a family history of breast cancer, have failed to show such a benefit (Powles et al., 1998; Veronesi et al., 1998). The efficacy of Tamoxifen among BRCA1 carriers has been especially argued because their tumours are more likely to be oestrogen receptor-negative.

With those patients already affected, more radical surgical treatment is considered, for example uni- or bilateral mastectomy is performed in 12 of 16 European centres on women whom breast-conserving surgery would be otherwise appropriate (Vasen et al., 1998). Prophylactic mastectomy and oophorectomy are topics of discussions with women having significantly increased risk. In some countries, prophylactic surgery has been well accepted; for example in the Netherlands, 51% of healthy mutation carriers opted for bilateral prophylactic mastectomy and 64% for bilateral prophylactic oophorectomy. In particular young women with children opted for mutation testing and prophylactic mastectomy (Meijers-Heijboer et al., 2000).

Most recommendations nowadays lack a strong scientific basis. The effect of prophylactic mastectomy is clear, but what has not been studied is how effective it is compared to other preventive measures (Hartmann et al., 1999; Meijers-Heijboer et al., 2001). While such results in incidence reduction are promising, further studies are urgently needed, and high hopes exist for less severe interventions such as chemoprevention.

Aims of the study

The general purpose of this study was to identify families with a hereditary predisposition to breast cancer and to study clinical features of those families.

The specific aims were to evaluate:

1.The frequency of breast cancer patients having a family history of breast and/or ovarian cancer and the number of their relatives affected

2. The number of families with a possible hereditary predisposition to breast cancer and the number of individuals at risk in those families

3. The validity of the family history of breast and ovarian cancer reported by the breast cancer patient

4. The standard life-table method in comparing the breast cancer risk of families with multiple cases of breast cancer to that of the general population

5. The risk of cancer in Finnish BRCA1 and BRCA2 mutation-positive and mutation-negative families

6. The survival of breast cancer patients belonging to breast cancer families

Patients and methods

Collection of probands and ascertainment of family history

During the year 1993, we collected two series of patients; young breast cancer patients (diagnosed before age 40), and patients with bilateral disease, all of whom had been diagnosed from 1985 to 1993 at the Department of Oncology, Helsinki University Central Hospital (HUCH). Of the 348 surviving patients receiving our family history questionnaires, 288 (83%) replied. Of these, 170 had been diagnosed before age 40, and 123 had bilateral breast cancer, with 5 fulfilling both criteria and being included in the series of young patients.

Since the year 1993, we have asked about family history of cancer systematically among all breast cancer patients at the Department of Oncology. First, to reach all prevalent breast cancer patients visiting the Department of Oncology, we asked the family history of such patients during one year from 10 November, 1993 to 10 November, 1994. During the following years, all new patients were interviewed. At the time of Study IV (1998) we had interviewed a total of 2 670 unselected patients (Figure 3).

All breast cancer patients first received a one-page questionnaire (Appendix 1), and if there was reason to believe that our selection criterion was fulfilled, a more detailed family history questionnaire was sent, or each was interviewed by telephone.

Family identification

One uniform selection criterion identified our breast cancer families. This was: at least three first- or second-degree relatives in the family (including the proband) with breast or ovarian cancer, irrespective of age. Male relatives were excluded in calculating the degree of relationship for the criterion. This criterion was selected in order to reach all breast cancer cases having a familial background, not just high-risk or early-onset cases. Six families fulfilling our criterion were referred from other hospitals in southern Finland (Figure 3).

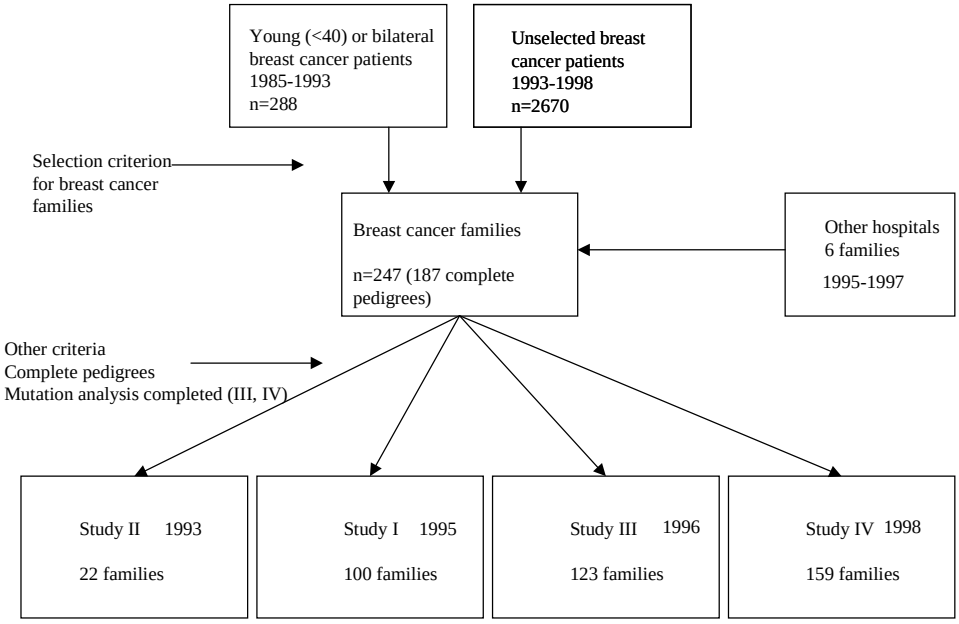

Figure 3. Ascertainment of families from the Department of Oncology and from other hospitals. Number of patients and families interviewed and selected for the studies at different time periods.

Pedigree construction

The genealogy of the families was confirmed through church parish registries and the Population Register Centre. Population registration in church registries in Finland dates back to the 16th century. We traced families as far back as the parents of the earliest known breast/ovarian cancer generation, with the most distant persons having been born at the end of the 18th century. Subsequently, information was collected on concerning all descendants. At the time of Study IV, we had a total of 187 complete pedigrees. In Study IV, we traced in some of the families only the first-degree relatives of the breast cancer patients. Information from church parish registries included offspring, personal identification numbers, and dates of death or emigration. Personal identification numbers (personal identifiers, PID) have, since 1967, been given to all residents of Finland, and after obtaining these personal numbers, we acquired dates and causes of death from the Statistics Finland office and Population Register Centre, as well.

Verification of cancer diagnoses

Cancer diagnoses of the patients and of all relatives traced, including those not reported as having cancer, were confirmed through the Finnish Cancer Registry and through hospital records of the Department of Oncology, HUCH. Causes of death were also registered, when possible.

In Finland, a nation-wide cancer registry was founded in 1952, and registration began in 1953. All hospitals, physicians, and pathology laboratories are required to notify the Finnish Cancer Registry (FCR) of all cancer cases that come to their attention. The FCR also receives information from all death certificates mentioning a cancer diagnosis. The completeness of the FCR for solid tumours is over 99% (Teppo et al., 1994). Our patients' family members who had PID were followed up for cancer incidence by a PID-based automatic record linkage. Some of the families were also manually linked to the Cancer Registry by name and date of birth.

Genetic analysis in Studies III and IV

At first 100 patients were screened for germ-line mutations in the coding regions and splice boundaries of the BRCA1 gene by the HA/SSCP technique for exons 2-10 and 12-24 and by PTT for exon 11, and of the BRCA2 gene by the HA/SSCP technique for exons 2-9 and 12-27 and by PTT for exons 10-11. Of the DNA samples, 70 were also screened at Myriad Genetic Laboratories, Salt Lake City, Utah, for BRCA1 mutations by direct sequencing through the coding regions and splice sites (Vehmanen et al., 1997 a; Vehmanen et al., 1997 b). In the remaining families, allele-specific oligonucleotide (ASO) hybridisation or restriction fragment length polymorphism (RFLP) was used to study all known Finnish BRCA1 and BRCA2 mutations. This analysis was further complemented by a protein truncation test (PTT) of BRCA1 exon 11 and BRCA2 exons 10 and 11 in families including young patients or ovarian cancer patients. Mutations identified were confirmed by direct DNA sequencing (Sarantaus et al., 2000; Vahteristo et al., 2001). No large deletions or other chromosomal rearrangements have been observed in an extensive analysis of 80 Finnish breast and/or ovarian cancer families (Lahti-Domenici et al., 2001). Thus, we believe that most of our non-BRCA1/2 patients are true non-carriers.

Ethics

The ethics committees of the Department of Oncology and the Department of Obstetrics and Gynaecology, HUCH, and of the Ministry of Social Affairs and Health in Finland have given the permission for this study. Blood samples were obtained from probands and family members willing to participate in the genetic analysis. Written informed consent was obtained at the time of sample donation. Relatives were not contacted without the permission of the proband, and no information on anyone's diagnoses was given to any relatives. Results of the mutation analysis were not revealed to the families. All families with a suspected hereditary disposition were offered genetic counselling. If they wished to have molecular genetic analysis it was discussed at the genetic counselling sessions at the Department of Clinical Genetics, and if sufficiently high probability existed of a mutation, analysis followed.

Evaluation of family history reported by patients (I)

Three series of patients were included. The first two series comprised young patients or bilateral patients (N=288, Figure 3). The third series comprised the unselected patients (in terms of age and laterality) (N=1282), which were systematically interviewed from November 1993 to May 1995.

Our family history criterion was fulfilled by 16 young cases, 11 bilateral cases, and 92 unselected cases. These series were partly overlapping, because 14 of these young or bilateral patients were visiting our clinic during the collection of unselected patients. Five cases (four unselected and one bilateral) were excluded, as their information had already proved to be incorrect at the beginning of the study, and thus those families did not fulfil the criterion.

Thus we identified a total of 100 families that were included in further analysis, i.e., complete pedigrees were drawn and cancer diagnosis confirmed. We compared the data obtained from registries to data reported by patients in order to assess the validity of reported cancers. We determined the number of potential healthy candidates who would

need genetic counselling and presymptomatic screening in 99 families, with verified family and cancer diagnosis data. One family was excluded because many of the family members emigrated. Potential candidates had to be alive, be between 20 and 70 years old, and be first-degree female relatives of breast or ovarian cancer patients. For the analysis, cancer information was updated to June 1997, and ages of relatives were calculated for that same year. Confidence intervals for proportions are based on ”exact” confidence limits based on binomial distribution.

Comparing risk of cancer by use of the standard life-table method (II)

In the series of young or bilateral patients a total of 26 families were identified. At the time of Study II, we were able to draw a pedigree with reliable data on chronological age and age at breast cancer onset for relatives of 22 families. In these 22 families, a total of 77 women had breast cancer. All first-degree female relatives of breast cancer patients in the 22 pedigrees were selected for the life-table analysis. This meant 211 individuals ranging in age from 0.1 to 92 years. In our analysis, we could compare their breast cancer risk as a function of age at onset to that of the general population.

The age-specific incidence rates for breast cancer came from the Finnish Cancer Registry (1993). The population incidence data were put into a BMDP statistical program (Dixon, 1988). For practical reasons, these incidence data were then compressed into a sample of 1 000 cases comprising 892 women not developing breast cancer by the age of 85 years and 108 women developing breast cancer at various ages. For each 10-year cohort, we coded the same percentage of cases as in the population incidence data. The mean age of each cohort was recorded as age of onset for all cases in each cohort.

The data were analysed with the BMDP Statistical Software in the VAX/VMS system. Age at onset was calculated by the Kaplan-Meier product limit estimate. The significance of age at onset was determined by the log rank test, which gives equal weight to all observations. The life-table method was used to estimate the breast cancer hazard function. Minimizing the evident selection bias in comparing the families with the general population required four different analyses. (1) Those persons not having breast cancer were included or (2) they were excluded from the analysis of the cumulative probability, (3) The hazard function were based only on individuals who eventually developed breast

cancer, or (4) In the fourth and most conservative approach, all affected individuals used to identify the cancer families were excluded: after this exclusion, only 14 breast cancer cases were left for analysis.

Estimation of cancer risks among BRCA1, BRCA2, and non-BRCA1/2 families (III)

At the time of Study III, we were able to draw a pedigree with reliable data on all siblings in nuclear families with complete follow-up time for a total of 123 families. Probands had been diagnosed between 1985 and 1996. Blood samples were obtained from 107 (87%) probands who gave their written informed consent; 16 of the probands either refused, or they failed to give blood samples. Altogether, 10 different BRCA1 mutations were identified in 12 families, and 5 different BRCA2 mutations in 11 families. In the remaining 84 families, no mutations were detectable.

We traced families backwards as far as the parents of the earliest known cancer generation. We then traced all their descendants (N=7115) through the parish registries, until their death or until further offspring were considered unlikely (female age 55, male age 70). Before PID were given (1967), 686 of the relatives were deceased and 85 had emigrated. Thirty-five relatives (0.5%) were lost from follow-up before they died or obtained PIDs. The tracing of PIDs was thus successful for 6 309 family members (Table 5). For them, dates and causes of death, and dates of emigration came from the Statistics Finland and the Population Register Centre.

Expected numbers of cancer cases were based on person-years at risk and the population-based gender-, age-, and calendar period-specific incidence rates for the general population. The standardized incidence ratios (SIRs) were calculated by dividing the observed numbers of cancer cases by the expected ones. The 95% confidence intervals (95%CI) for the SIRs were calculated, assuming that numbers of observed cases followed a Poisson distribution. The analyses were stratified by the mutation status of the family and by degree of relationship to the patients. In the BRCA1 and BRCA2 families, degree of relationship was determined to the nearest breast or ovarian cancer patient (the putative mutation carriers) and in the non-BRCA1/2 families to the nearest breast cancer patient only.

Table 5. Numbers of persons (N) and person-years (P-Y) by BRCA status of the families

	BRCA1 (12 families)				BRCA2 (11 families)				non-BRCA1/2 (84 families)			
	females		males		females		males		females		males	
	N	P-Y	N	P-Y	N	P-Y	N	P-Y	N	P-Y	N	P-Y
All relatives	338	7 638	303	7 183	438	9 604	389	8 969	2 553	57 393	2 288	55 047
Relationship*												
First-degree	113	2 375	89	2 268	125	2 772	100	2 434	658	13 906	478	12 273
Second-degree	113	2 721	102	2 465	131	3 120	140	3 473	686	16 486	656	16 702
Third-degree	112	2 542	112	2 450	182	3 712	149	3 062	1 209	27 001	1 154	26 072
Age**												
0-14	191	2 104	193	2 206	310	3 490	266	2 697	1 497	16 440	1 496	16 345
15-29	66	2 267	62	2 175	44	2 740	64	2 694	372	15 936	394	16 492
30-44	34	1 705	20	1 555	51	1 760	39	2 138	231	11 041	227	11 958
45-59	32	903	22	838	23	957	8	978	245	7 234	100	6 586
60-74	13	482	2	326	9	556	10	387	164	4 665	61	2 923
75+	2	177	4	82	1	99	2	77	44	2 077	10	743
Breast cancer patients***	37	306	-	-	49	327	-	-	272	2176	-	-

*Relationship to (nearest) breast or ovarian cancer patient in the BRCA1 and 2 families and to (nearest) breast cancer patients in the non-BRCA1/2 families
**at beginning of follow-up
***patients included in all family members

The family members were followed up for cancer incidence by a PID-based automatic record linkage with the Finnish Cancer Registry. The follow-up started on 1 January, 1967 or at birth (whichever came later) and ended either at a person's death, date of emigration, or 31 December, 1997 (whichever came first). However, for the proband, and for the other breast or ovarian cancer patients reported by the proband, any follow-up years before the date of their breast or ovarian cancer diagnosis were excluded, because by definition they could not have had fatal cancers before that. In the analysis of risk of subsequent cancers among all the breast cancer patients, the follow-up always started from the date of first breast cancer diagnosis.

The analysis concerning risk of ovarian cancer among relatives was limited to families with at least three first- or second-degree relatives with breast cancer, irrespective of ovarian cancer in the family. Those ten families that were originally identified in part because of existence of ovarian cancer cases were excluded. The numbers of persons in the BRCA1, BRCA2, and non-BRCA1/2 families in this analysis were 186 (4 374 person-years), 438 (9 650), 2 416 (54 503), respectively.

In order to compare breast cancer risk in BRCA1, BRCA2, and non-BRCA1/2 families, the probands were excluded, and the SIRs were calculated in two ways: (1) All other a priori known breast cancers were excluded or (2) all of these were included. Thus, the SIRs give the lowest and highest possible estimate of true relative risk, which cannot be estimated exactly in this setting. The number of persons (and person-years) in this higher-estimate analysis in the BRCA1, BRCA2, and non-BRCA1/2 families were 327 (7 816), 427 (9 788), and 2 470 (59 304), respectively.

Kaplan-Meier analysis was used to calculate the cumulative incidence of breast and ovarian cancer (SPSS for Windows, 1998). Death and loss from follow-up were considered as censored observations. Median ages at cancer diagnoses were based on cumulative incidence rates.

Assessing the survival of breast cancer patients (IV)

A total of 159 families with probands diagnosed between 1985 and 1998 were included in Study IV. Families were included if a blood sample and also a written informed consent were obtained from at least one breast or ovarian cancer patient in each family. Furthermore, at least one patient in each family had to be diagnosed between 1953 and 1995, i.e., when cancer registry records were available. To confirm the cancer family history of the non-BRCA1/2 families, at least three cases had to be confirmed through the FCR, or if breast cancer was diagnosed before the founding of the FCR in 1953, confirmed by the death certificate. Twelve different BRCA1 mutations in 14 families, and 6 different BRCA2 mutations in 15 families were detected, but in the remaining 130 families, no mutations.

Exceptionally, the pedigrees of some of the newer families (64), were mainly based on interview data, but PIDs of all the reported breast cancer patients were uniformly traced and cancers confirmed through the FCR.

The 159 families initially included 632 breast cancer patients. Only those 432 patients who had their first primary breast cancer diagnosed from 1953 to 1995 and who had no former malignancies were accepted. Probands from the newer families (64) were

diagnosed after the year 1995. We further excluded 73 probands diagnosed during the study period whose time-interval from date of diagnosis to date of interview or blood sampling exceeded 6 months, to avoid selection bias towards long-term survivors. Thus, a total of 359 patients from these breast cancer families were included in the survival analysis. For a comparison group, all other breast cancer patients diagnosed with their first primary breast cancer, without former malignancies, during the time period from 1953 to 1995 were extracted from the FCR (N=59 881). (Patients diagnosed at autopsy or whose diagnosis was based on death certificate only were excluded.) Follow-up of all the patients started at the date of diagnosis and ended on the date of death, of emigration or on the study's closing day (31 December, 1997), whichever occurred first.

Cumulative relative survival rates (RSR) were calculated, as the ratio of observed to expected survival rates, by a computer program package designed specifically for this purpose (Voutilainen et al., 1998). Expected survival rates were derived from the sex-, age-, and calendar year-specific life-tables of the general Finnish population; 95% confidence intervals were calculated by Greenwood's formula (Cutler and Ederer, 1958).

To assess the size of the potential bias resulting from inclusion of those probands (N=22) interviewed within 6 months from diagnosis, we performed the analyses also without them. Further, non-BRCA1/2 cases were analysed without cases from those families not screened for the whole coding sequences but screened mainly for known mutations only, in order to exclude possibly undetected BRCA1/2 cases. Patients diagnosed from 1953 to 1966 were analysed separately from those diagnosed from 1967 to 1995 to avoid potential selection bias, because individuals who died before acquiring a PID might have been missed in the manual search of the FCR files, thus introducing a potential (although minimal) bias towards longer survival times.

To obtain estimates of relative excess risk of death (RR) between patients in the breast cancer families and those with sporadic breast cancer, we used the GENMOD procedure in the SAS statistical package to produce a discrete proportional hazards model for the first 5 years of follow-up (SAS Statistical Software, 2000). A model was constructed including the following prognostic factors: age, stage as recorded in the FCR, and year of diagnosis as well as mutation status of the family.

Results

Number of families and individuals at risk (I)

Family history of breast or ovarian cancer was reported by 27 to 36% of the patients, and 9.4% (95% CI 5.5-14.8) of the young, 8.5% (95% CI 4.2-15.0) of the bilateral, and 6.9% (5.5-8.4) of the unselected patients fulfilled the criterion of at least three affected patients with breast or ovarian cancer in the family (Table 6*)*. Among the families fulfilling the criterion, ovarian cancer cases were present in 19 to 23%. Among the 88 families of the unselected patients, bilateral breast cancer cases were found in 23 (26%).

Table 6. Family history: number of families (%) in series of young, bilateral, and unselected patients; inheritance through a male accounted for by excluding male relatives when calculating degree of relationship

Serie		Number of breast or ovarian cancer cases in families (proband included)					
	Total	≥5*	≥4*	≥3*	2*	>1*	1**
Young(<40y)	170	5 (2.9)	10 (5.9)	16 (9.4)	30 (17.6)	46 (27.0)	124 (72.9)
<35y	59	1 (1.7)	2 (3.4)	7 (11.9)	12 (20.3)	19 (32.2)	40 (67.8)
Bilateral	118	2 (1.7)	5 (4.2)	10 (8.5)	32 (27.1)	42 (35.6)	76 (64.4)
Unselected	1282	16 (1.2)	42 (3.3)	88 (6.9)	281 (21.9)	369 (28.8)	913 (71.2)
<35y	74	2 (2.7)	4 (5.4)	9 (12.1)	13 (17.6)	22 (29.7)	52 (70.3)
<40y	182	5 (2.7)	9 (4.9)	16 (8.8)	32 (17.6)	48 (26.4)	134 (73.6)
<50y	564	12 (2.1)	25 (4.4)	45 (8.0)	132 (23.4)	177 (31.4)	387 (68.6)
Bilateral	112	3(2.7)	4 (3.6)	9 (8.0)	33 (29.5)	42 (37.5)	70 (62.5)
≥40y/unilat	988	8 (0.8)	29 (2.7)	63 (6.4)	216 (21.8)	279 (28.2)	709 (71.8)
<40y/unilat	169	5 (3.0)	9 (5.3)	16 (9.5)	28 (16.6)	44 (26.0)	125 (73.9)

Data in families with less than 3 cases is based on patient reports
*first- or second-degree relatives ** no first- or second-degree relatives
(Young and bilateral series partly overlapping with unselected)

Potential healthy relatives at risk and thus being candidates for genetic counselling and presymptomatic cancer screening were determined in 99 families fulfilling the criterion (Table 7). For the breast or ovarian cancer patients, there were 307 healthy first-degree female relatives (age 20-70) in the families, 3.1 females per family.

Table 7. Potential female candidates for genetic counselling, diagnostic testing, or presymptomatic screening

Cases and relatives in 99 families	Females	Females/family
Breast or ovarian cancer cases	369	3.7
First-degree female relatives (healthy)	404	4.1
age 20-70	307	3.1
35-70	233	2.4
35-50	110	1.1

Accuracy of family information reported by patients (I)

The accuracy of the information reported by the patients was studied in 100 breast cancer families identified at that time (Table 8). A total of 272 breast and ovarian cancer diagnoses of relatives were obtained in these families, including cases reported by the patients that were not possible to be confirmed, but omitting those reported incorrectly (details below). Of the 272 cases, 35 of the diagnoses included were not known by the proband and were found only from registries. Our probands were able to report 100% of their first-degree relatives affected with breast or ovarian cancer, 99% of the second-degree relatives, but only 50% of the third- to fifth- degree relatives.

Table 8. Relatives with breast or ovarian cancer reported by patients: Accuracy of patient reports

Relationship of cases to patient (degree)	Total N of cases in families*	N of cases reported by patients	Cases reported correctly	% of cases reported (sensitivity)	% of cases reported correctly
First	94	99	94	100	95
Second	110	114	109	99	96
Third	49	32	30	61	94
Fourth to fifth	19	4	4	21	100
Total	272	249	237	87	95

*unconfirmed cases included

The proband correctly identified 237 (95%) of 249 reported primary sites (leaving 12 (5%) incorrect diagnoses). However, the five families excluded at the beginning of the study included an additional ten reported affected relatives, and five of these were incorrect. Hence the actual percentage of incorrect diagnoses was 7%. Table 9 shows the 24 unconfirmed diagnoses reported by the patients; 22 cases were lost from follow-up. Two of the reported ovarian cancer cases were actually diagnosed as undefined abdominal cancers.

Table 9. Incorrectly reported or unconfirmed cases

Total number of reported diagnoses	249
Incorrect or unconfirmed diagnoses	36 (14%)
Incorrect diagnoses	12 (5%)
non-existent malignancy quoted	4 (2%)
mistaken site of malignancy	8 (3%)
Unconfirmed diagnoses	
pathology report unclear	2
lost from follow-up	22 (9%)
abroad	12
died before 1953*	4
information lost or innaccurate	6

*before founding of Finnish Cancer Registry

Standard life-table method in risk estimation for families (II)

When the breast cancer-free survival of the relatives of young and bilateral probands was directly compared with Kaplan-Meyer analysis to that of the general population, the difference was large. The cumulative probability of living without breast cancer is presented in Table 10. However, when the two affected relatives who defined the family were excluded, only the relatives of the young probands showed a significantly lower age of onset than did the population (median 51 vs. 63, p=0.0002, log rank). The breast cancer hazard function showed a similar tendency. When we included in the analysis only those patients who eventually developed breast cancer, the hazard was much greater for the relatives of young probands but among relatives of bilateral (and older) probands was similar to that of general population.

Table 10. Number of breast cancer cases and cumulative probability of avoiding breast cancer by age in first-degree female relatives in families of young probands, bilateral probands, and the general population. Probands are excluded.

Age-range (years)	Families of young probands (n=13)		Families of bilateral probands (n=9)		Population/1000	
	N of breast cancer cases	Cumulative risk	N of breast cancer cases	Cumulative risk	N of breast cancer cases	Cumulative risk
30-39	3	0.97	2	0.97	3	1.0
40-49	6	0.90	4	0.89	15	0.98
50-59	10	0.74	5	0.78	24	0.96
60-69	9	0.54	4	0.68	21	0.94
70-79	4	0.39	3	0.57	21	0.92
80-89	1	0.28	4	0.27	24	0.87
Total	33		22		108	

By the standard life-table method, the overall risk of breast cancer in these families, all first-degree relatives included, was 7.6-fold ($p<0.0001$, log rank) higher than in the general population. If the two affected relatives who defined the families were excluded, the overall risk was still 2.8-fold ($p=0.0002$) higher. For relatives of young probands it was 3.5 ($p<0.0001$) and for relatives of bilateral breast cancer probands it was 1.8 ($p=0.2$). When we calculated the risk of breast cancer as a function of age at onset for only those relatives who eventually developed breast cancer, the risk was 1.7-fold ($p=0.0005$) in all families pooled; in the families of the young probands 2.0-fold ($p<0.0001$), and in the families of the bilateral probands 1.3-fold ($p=0.1$).

Risk of cancer among patients and family members (III)

Ovarian cancer

The relative risk of subsequent ovarian cancer, following the first breast cancer, was very high in the BRCA1 families (SIR 61) and in BRCA2 families (SIR 38), whereas no cases occurred in our non-BRCA1/2 families (Table 11). The cumulative incidence of ovarian cancer following breast cancer by the age of 70 was 29% (95% CI 8-51%) in the BRCA1 and 8% (0-20%) in the BRCA2 families (Figure 4A).

Similarly, a high relative risk of ovarian cancer among the first-degree relatives of breast cancer patients was found in both BRCA1 (SIR 29) and BRCA2 (SIR 18) families, but no elevated risk appeared in the families without these mutations (Table 11). The cumulative risks of ovarian cancer among the first-degree relatives by the age of 70 was 25% (95% CI 4-45%) in BRCA1 families and 12% (2-21%) in BRCA2 families (Figure 4B).

Table 11. Risk of breast and ovarian cancer among breast cancer patients (subsequent cancer following first primary breast cancer) and among female relatives in BRCA1, BRCA2 and non-BRCA1/2 families. Observed (obs) and expected (exp) numbers of cases and standardized incidence ratios (SIR) with 95% confidence intervals (CI)

	BRCA1				BRCA2				non-BRCA1/2			
	obs	exp	SIR	95% CI	obs	exp	SIR	95% CI	obs	exp	SIR	95% CI
OVARIAN CANCER RISK												
Among breast cancer patients	5	0.1	61	20-142	4	0.1	38	10-98	0	0.9	0.0	0.0-4.2
Among relatives*												
first- degree	5	0.2	29	9.4-68	9	0.5	18	8.4-35	3	3.1	1.0	0.2-2.9
second-degree	2	0.2	11	1.3-39	-	0.3	-	0.0-13	1	1.9	0.5	0.0-3.0
third-degree	1	0.2	6.1	0.2-34	-	0.1	-	0.0-32	4	1.9	2.2	0.6-5.5
all relatives	8	0.5	15	6.6-30	9	0.9	10	4.6-19	8	6.8	1.2	0.5-2.3
BREAST CANCER RISK												
Among breast cancer patients	5	0.5	11	3.6-26	5	0.5	10	3.3-24	16	4.3	3.7	2.1-6.1
Among relatives**												
first-degree	12-22	1.9	6.3-12	3.3-18	13-26	2.4	5.4-11	2.9-16	27-132	16	1.7-8.4	1.1-9.9
second-degree	0-1	1.1	0-0.9	0.0-5.1	7	1.2	5.7	2.3-12	10-27	9.3	1.1-2.9	0.5-4.2
third degree	4-5	0.9	4.4-5.5	1.2-13	2	0.4	4.8	0.6-17	5-13	8.6	0.6-1.5	0.2-2.6
all relatives	16-28	3.9	4.1-7.3	2.3-11	22-35	4.0	5.5-8.8	3.4-12	42-172	34	1.2-5.1	0.9-5.9

*within 97 families chosen by selection criterion: at least three first or second degree relatives with breast cancer
** The range of the risk: lower value excludes all breast cancers used as the basis of cohort selection, and the higher value includes all of them; 95% CI based on the lower confidence limit of the lower estimate to higher limit of the higher estimate.

Breast cancer

The risk of subsequent breast cancer was equally high among breast cancer patients in BRCA1 and BRCA2 families (SIR 10.9 and 10.1, respectively) but lower in families without mutations (SIR 3.7) (Table 11). The cumulative incidence of subsequent breast cancer by age 70 among breast cancer patients was 25% (95% CI 6-44%) in BRCA1 families, 25% (5-46%) in BRCA2 families, and 8% (4-12%) in families without mutations (Figure 4C).

The relative risk estimates for breast cancer in the BRCA1 and BRCA2 families were similar and somewhat lower in the non-BRCA1/2 families (Table 11). The cumulative incidence in the BRCA2 seemed to exceed the others due to a cluster around the age of 55 (Figure 4D). No male breast cancer cases were seen (expected number 0.17).

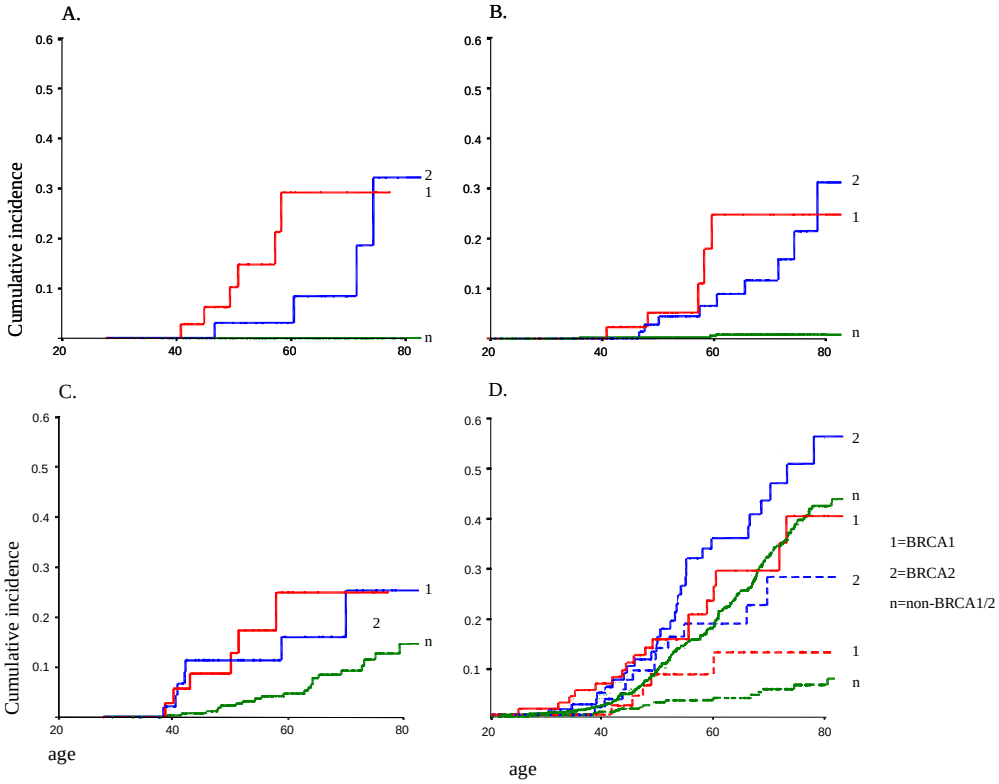

Figure 4. Cumulative incidence of cancer in BRCA1, BRCA2, and non-BRCA1/2 families
A. Subsequent ovarian cancer among breast cancer patients.
B. Ovarian cancer among first-degree relatives of breast cancer patients (among 97 families selected solely due to breast cancer).
C. Subsequent breast cancer among breast cancer patients.
D. Range of breast cancer among first-degree relatives: in lower curves (dotted lines) all a priori known breast cancers reported by the proband are excluded, and in higher curves these are included (only the proband is excluded).

Other cancers

The SIRs for cancers other than breast and ovary among the first-degree relatives of breast or ovarian cancer patients in mutation-positive families, and of breast cancer patients in

mutation-negative families were close to one. The only significantly elevated relative risk was that of prostate cancer in the BRCA2 families (SIR 4.9, 95% CI 1.8-11). The SIR was highest, 12.3 (0.3-68.3), in the age-group 45 to 59 years and decreased with age, being 5.5 (1.1-16.1) and 3.3 (0.4-11.8) in the age-groups 60 to 74 and 75 years or over, respectively.

Survival of breast cancer patients (IV)

The patients in the BRCA1 and BRCA2 families were younger than those in the non-BRCA1/2 families or the sporadic patients. The proportion of non-localized tumours in BRCA2 families was higher (56%) than in the other categories (39-44%). The proportion of medullary carcinomas among the patients in the BRCA1 families was 13% (based on four cases) but only 1% among the sporadic cases. Otherwise, the distributions by stage, calendar period of diagnosis, and histology of tumours were fairly similar among the different patient groups.

Patients in the BRCA1 families tended to have the worst outcome in most strata of the prognostic factors. Their overall 5-year relative survival rate (RSR) was 67% (95% CI 48-86%) compared to 78% (95% CI 77-78%) for the sporadic cases. The 5-year RSR for patients with localised tumours was 78% (95% CI 54-103%) among BRCA1 families and it was 95% (95% CI 94-95%) for the sporadic cases. The relative risk of excess death (RR) among all patients in the BRCA1 families was 1.30 (0.63-2.70) when compared with that for patients with sporadic tumours (Table 12).

The overall unadjusted survival rate of patients in the BRCA2 families did not differ from that of the sporadic ones. However, their cancers were more advanced and, as a consequence (i.e., after adjusting for stage), their RR was lower (0.78, 95% CI 0.39-1.57) than that of the sporadic cases (Table 12). The survival of patients among non-BRCA1/2 families was similar to that of the sporadic breast cancer cases. The RR was 1.02, 95% CI 0.75-1.39 (Table 12).

Table 12. Relative risk (RR) of excess death within 5 years after diagnosis adjusted for stage, age of diagnosis, period of diagnosis, and follow-up year

Prognostic factor	N_p	N_d	RR	95% CI
Family history				
BRCA 1	32	12	1.30	0.63-2.70
BRCA2	43	12	0.78	0.39-1.57
Non-BRCA1/2	284	77	1.02	0.75-1.39
Sporadic	59 517	20 580	1.00	Ref

N_p = Number of breast cancer patients at the beginning of the follow-up

N_d = Number of deaths during the entire 5 year follow -up time

The exclusion of the probands interviewed within 6 months from diagnosis did not affect results. Nor did the exclusion of the BRCA1/2-negative families, which were screened for known mutations only, affect the results.

Discussion

Breast cancer is a heterogeneous disease whose aetiology, for the most part, is unknown. Still, for a long time, it has been known to "run in families", and family history of breast cancer is known to be one of the strongest risk factors for the disease. During the last decade the hereditary background of the disease has been under intense research. Two high-risk breast cancer-predisposing genes have been found, and this has led to further research into the prevalences of the mutations and phenotypes linked to these genes. Moreover, because a large number of breast cancer families are not explained by mutations in the BRCA1 or BRCA2 genes, studying this group of families will be a challenging task in the future.

Genes and environmental factors predisposing to cancer may vary from one population to another. The Finnish population in particular may differ from others because it has been formed from a small founder population and has lived in isolation as a result of geographical and linguistic factors. There is thus a need to evaluate the role of genetic factors in Finland as well, identify the families, quantify the risks more precisely, and study other clinical features to allow for appropriate counselling, surveillance, and management.

Finnish population registration and cancer registration offered us exceptional facilities to construct full pedigrees with reliable cancer data. Currently nearly all cancer patients (99%) with solid tumours are registered in the Finnish Cancer Registry (Teppo et al., 1994), and population registration in church registries in Finland reaches back to the 16th century. Thus, unlike many other researchers, we did not have to rely on information reported by the patients, and reliable information about diagnoses and ages of onset could be obtained. Additionally, this gave us the advantage of an opportunity to study the reliability of patient's reports of their family history.

Identification of the families

In the present study, family background of breast cancer among unselected breast cancer patients appeared relatively common, as about 29% (95% CI 26-31) of the patients reported at least one first- or second-degree relative with breast or ovarian cancer. This is in agreement with other studies showing 27 to 35% of patients with a reported family history of breast cancer among such relatives (Sattin et al., 1985; Lynch et al., 1988; Thompson, 1994). Recently, in a Swedish population-based study, almost half (48%) of breast cancer patients under 41 reported a family background in first- or second-degree relatives (Loman et al., 2001).

However, it is not easy to distinguish between genetic and other clustering of cancer cases. Studying identical and non-identical twins allows the comparison of influence of environment and genetic factors. A large twin study (Lichtenstein et al., 2000) combining information from twin registries from Denmark, Finland, and Sweden showed that inheritable factors play a role in 27% (95% CI 4-41) of the breast cancers. Earlier, the estimations have been lower, and genetic factors were thought to play a role in only some 5 to 19% of the breast cancers (Colditz et al., 1993; Slattery and Kerber, 1993; Madigan et al., 1995).

Our criterion of three or more affected first- or second-degree relatives identified 7% of our unselected patients into breast cancer families. The proportion was higher among young patients, and about 9% of the patients under 40 had such a family background. This proportion is a little lower than detected in Sweden by Loman et al. (14%) among patients under 41 (Loman et al., 2001). In a previous family history study from Finland, of 669 patients, 10 (2%) reported a possible hereditary background, which was defined as at least three cases among first-degree relatives (Doepel et al., 1995). We found exactly the same figure in our study if we used the same criterion and allowed inheritance only from the maternal side. If we defined families with another more strict criterion of four or more affected first- or second-degree relatives, the proportions would be 3 to 6%, depending on the series of patients. It is not easy to compare our result to earlier ones, since different criteria for hereditary or familial breast cancer are used in almost every study. Furthermore, we cannot claim that our criterion only identifies families with a truly

hereditary predisposition. Still, the result quantified the families with a family history strongly suggestive of genetic predisposition, and which were obviously already worried about their family history, and these make up probably the major fraction of families in need of genetic counselling. Criteria for testing the mutation in the family as well as for presymptomatic screening and clinical trials comprise a complex issue and need to be carefully considered.

Correct identification of at-risk families and their relatives is important for their counselling, diagnostic testing, and clinical management. Accurate family history forms the basis of all this. Confirmation of the diagnosis of the relatives is usually considered necessary in clinical management, although it is laborious. In this study, the information reported by the patients proved to be very accurate. Only about 5 to 7% of all reported diagnoses among breast cancer families was incorrect. However, we consider verification of the cancers important when making decisions about patient management based on family history. Diagnostic testing, intensive surveillance, chemoprevention, or even prophylactic operations may extensively affect the quality of life of these women, and should be based on the most reliable information available.

In the present study, in contrast to cancer cases among first-degree relatives, cases among distant relatives: third- (e.g., cousins) and fourth-degree relatives, were not well known by the probands. They knew only 60% of cancer cases among their third-degree relatives. However, this information on cancers of third-degree relatives may also be important for risk estimation in the family when considering diagnostic testing and cancer surveillance. Some families may be excluded from testing or screening procedures because of their lack of awareness of cancer cases in the family. Patients with a suspicious family history could be encouraged to discuss family cancers with their relatives.

The availability of diagnostic testing for BRCA1- and BRCA2-caused cancer predisposition has made it possible to identify carrier families and in those, the individuals at highest risk. Mutations in the known susceptibility genes BRCA1 and BRCA2 account for the majority of the early-onset families with several breast cancer patients and with ovarian or male breast cancer cases (Ford et al., 1998). However, often neither of these two genes explains breast cancer families, or the mutations cannot be found (Serova et al.,

1997; Vehmanen et al., 1997 b; Ford et al., 1998). In the mutation-negative cases, risk estimation remains solely based on family data.

Overall, because estimating the genetic susceptibility as well as the need for presymptomatic screening is based on family history, and it may be difficult, even with an accurate family history, to identify the high-risk families, we used the standard-life table method in order to assess their risk of breast cancer compared to that for the general population. Contrary to many other models or to simple criteria, this method takes into account also the numbers and ages of healthy family members. Among the families of young probands, the risk of breast cancer was significantly higher than in the general population, but the risk among relatives of bilateral (and older) probands compared to that of the general population was not statistically significant. Later, when the mutation analyses were completed, it appeared that five of the young probands were carriers of BRCA1 or BRCA2 mutations but none of the bilateral probands were.

Although the life-table method has not been studied in practise, it could prove to be a simple tool in everyday genetic counselling and be useful in selecting families and individuals for screening or prevention programmes among BRCA1- or BRCA2-negative individuals.

Risk of cancer, surveillance, and management

Breast cancer

Risk estimation of breast cancer among breast cancer families is difficult. The penetrance of BRCA1 and BRCA2 genes may be over-estimated because of ascertainment bias, as most of the studies are done with multiple cases of breast or ovarian cancer. Different mutations may have different penetrance, and if the study group is highly selected (for example some special ethnic subgroup), the modifying factors, genetic and environmental, may be clustered differently as well.

For the present study, the families were selected by use of a straightforward criterion of at least three first- or second-degree relatives with breast or ovarian cancer. This population

is probably the same as is usually referred to genetic counselling and risk-assessment. The criterion is looser than in many other family studies and allows inclusion of lower-risk families, as well. Furthermore, we could evaluate the risks among the mutation-negative families selected by the same criterion.

The risk analysis of cancers in the Finnish BRCA1 and the BRCA2 families, as well as in the mutation-negative families, revealed a high risk of breast cancer among all three groups. The risk analyses are sensitive to the ascertainment criteria of the families. It was thus impossible to assess the true breast cancer risk in the families, because existence of breast cancers strongly affected the basic selection of the families. Instead, we estimated the lowest and highest possible value of the rate, which allows valid comparison of the magnitudes of the risk among the three groups of families. The true value of the SIR of breast cancer lies between these two extremes: among first-degree relatives in BRCA1, 6.3 to 12, in BRCA2, 5.4 to 11, and in non-BRCA1/2 families, 1.7 to 8.4. Breast cancer in BRCA1 and BRCA2 families tended to manifest at younger ages than in non-BRCA1/2 families. However, the lifetime risk of breast cancer in mutation-negative families was similarly high. This is in line with other studies suggesting that other breast cancer predisposition genes exist besides BRCA1 or BRCA2 (Schubert et al., 1997; Serova et al., 1997; Ford et al., 1998; Kainu et al., 2000).

Although an excess risk of breast cancer among male carriers of BRCA2 mutations has been documented (Easton et al., 1997; Thompson et al., 2001), we found no affected men. This could, in principle, indicate other genetic or environmental factors modifying the risk of specific cancers in different populations. However, due to the rarity of male breast cancer, the present study can only exclude a relative risk exceeding 240.

In most centres in Europe (Eisinger et al., 1998; Vasen et al., 1998; Møller et al., 1999) as well as in clinical practise at the Helsinki University Central hospital, a breast follow-up is recommended for high-risk individuals by annual mammography, starting from the age of 30 to 35 years or five years earlier than the age of onset of the youngest affected family member. In our study (III) the risk of breast cancer started to rise considerably before 40 years of age both in mutation-positive and -negative families. This has also been documented elsewhere in BRCA1 and BRCA2 families (Ford et al., 1998). Thus, the timing of the surveillance seems reasonable and, based on this study, it is needed in the

high-risk families whether a mutation in a family is BRCA1, is BRCA2, or is not detected at all. Surveillance of the breasts among men seems not to be reasonable because of the lower risk detected in this and in previous studies (Easton et al., 1997; Thompson et al., 2001).

The number of healthy relatives at highest risk who could especially benefit from presymptomatic screening or preventive measures is not high, compared to the number in the population screening programs for specific age-groups already carried out in many countries with public health care systems. The number of women identified here in breast cancer families as being potentially at high risk was 1.1 females aged 35 to 50 years per family (Study II). For example, in Helsinki, with a population of 0.5 million, about 380 new breast cancer cases were affected in 1997 (Finnish Cancer Registry, 1997). Based on this study, 26 of these would be familial cases, and in their families would be 28 potential new screening candidates per year. This number would be cumulative in the first years, but slowly, with most of the families found, the females would belong to the same families. Altogether this is a small number compared, for example, to the 19 800 women aged 50 to 58 who were invited to mammography screening in 1997 in Helsinki. If the screening were extended to ages 20 to 70 years in these high-risk families, they would still total only 80.

However, the efficacy of mammography among younger women with high-density breast tissue is controversial (Kerlikowske et al., 1995) and has not been studied enough among young high-risk women. Another possibility for high-risk women, prophylactic mastectomy, is shown to be effective in preventing breast cancer. Recently, Hartmann et al. (1999) showed it to be associated with a reduction in incidence of breast cancer of at least 90%. Prophylactic oophorectomy has also been noticed to reduce the risk of breast cancer among carriers of BRCA1 mutations (hazard ratio 0.53) (Rebbeck et al., 1999). In Europe, these prophylactic surgeries are considered for proven carriers of BRCA1/2 mutations or for women with a lifetime risk of 1 in 4 or greater for breast cancer (Vasen et al., 1998; Evans et al., 1999). Based on this and previous studies, the risk among BRCA1 and BRCA2 families is high enough to initiate a discussion of prophylactic surgeries. Although it has been impossible to conduct randomised prospective trials comparing different techniques of prophylactic mastectomy, or comparing prophylactic mastectomy with other preventive measures, it seems to be the most effective method today. Among

non-BRCA1/2 families, the question of prophylactic mastectomy is more difficult, partly because of their somewhat lower risk than in mutation-positive families and because we cannot distinguish the true carriers of as-yet-unidentified genes.

In the present study, the cumulative incidence of subsequent breast cancer by the age of 70 was as high as 25% for patients from BRCA1 and BRCA2 families, while in the analysis by the Breast Cancer Linkage Consortium the corresponding risks were even much higher: 64% and 52%, respectively (Ford et al., 1994; Breast Cancer Linkage Consortium, 1999). The breast cancer patients in the non-BRCA 1/2 families had a less elevated risk of subsequent breast cancer, and their cumulative incidence by age 70 was about 8%. The SIR for subsequent breast cancer was 3.7. Although much lower than among mutation-positive families, these families' relative risk appeared higher than was the risk of subsequent breast cancer among all breast cancer patients in the general Finnish population (SIR 1.6) (Teppo et al., 1985). This result suggest recommending a more effective follow-up of the breast-cancer family patients or more radical management than in general. In accordance with this, most centres in Europe recommend total mastectomy (unilateral or bilateral) for breast cancer patients carrying BRCA1 or BRCA2 mutations (Vasen et al., 1998). Despite the obviously increased risk of contralateral breast cancer, some studies have suggested, however, no difference in the prognosis of patients treated with breast-conserving surgery and adjuvant therapy between patients with a family history or BRCA1/2 mutations or both and the sporadic control cases (Chabner et al., 1998; Eccles et al., 1999; Pierce et al., 2000).

Tamoxifen is effective in reducing the risk of contralateral breast cancer among the general population (Early Breast Cancer Trialists' Collaborative Group, 1998), but its effectiveness among BRCA1 and BRCA2 carriers has been disputed, especially among BRCA1 carriers, because their cancers are most often oestrogen-receptor negative. However, in a study by Narod et al. (2000), tamoxifen reduced the risk of contralateral cancer by 50% even among women with BRCA1 or BRCA2 mutations; combined with oophorectomy, the risk fell by over 80%. Tamoxifen may thus be effective even in preventing the first primary breast cancer among women at increased risk of breast cancer (Fisher et al., 1998).

Ovarian cancer

In BRCA1 families, a very high risk of ovarian cancer has been documented, with lower risks in BRCA2 families (Ford et al., 1998). The ovarian cancer risks in the present study were very high in both groups, with higher SIRs in the BRCA1 families. However, the higher risk appears to be attributed to the earlier onset of ovarian cancer in BRCA1 families. Among BRCA1 families the risk rose notably just after 40 years, with all cases diagnosed by the age of 60, while among BRCA2 carriers, the risk appeared to rise later and continued to rise until age 80. This difference in ages of onset is seen also in other studies (Risch et al., 2001). Johannsson et al. (1999) recently suggested that the increased risk of ovarian cancer among BRCA2 carriers may result from a selection bias. In our study, the ovarian cancer risk analyses were carried out in families selected on the basis of breast cancer cases only. Even had the analysis been made for the whole material, and all ovarian cancer diagnoses mentioned by the proband excluded (i.e., the follow-up was started at the date of diagnosis), the SIRs would have remained elevated (SIR 19, 95%CI 7.7-39.3, in BRCA1; and SIR 12.5, 95% CI 4.6-27.1, in BRCA2 families). It should also be noted that none of the BRCA2 mutations in the families studied here was located in the putative ovarian cancer cluster region of the gene, from which a higher ovarian cancer risk has been suggested (Gayther et al., 1997; Thompson et al., 2001). The risk of subsequent ovarian cancer was also very high among breast cancer patients in both BRCA1 (SIR 61, 95% CI 20-142) and BRCA2 (SIR 38, 95% CI 10-98) families.

The risk of cancer among mutation-negative families has not been studied, and one of the most important findings of this study was that the risk of ovarian cancer was not increased among the mutation-negative families. This is in accordance with results suggesting that almost all breast-ovarian cancer families are due to defects in the BRCA1 or BRCA2 genes (Ford et al., 1998). Based on our study, surveillance of the ovaries would not be necessary among non-BRCA1/2 families. However, in individual families, specific family history characteristics need to be taken into consideration, as mutation-detection schemes are not 100% effective.

In Europe, surveillance of the ovaries by gynaecological examination, sonography, and CA-125 is recommended in BRCA1 and BRCA2 carriers and in members of

breast/ovarian cancer families, and in some centers also in families with early-onset breast cancers (Vasen et al., 1998; Møller et al., 1999). However, the effectiveness of such surveillance is unknown. The other alternatives are chemoprevention and oophorectomy. It seems that for BRCA2 carriers the appropriate time for oophorectomy is during menopause, but this may be too late for BRCA1 carriers. Oophorectomy cannot completely prevent ovarian cancer, because some of the tissue of the ovaries is left in the peritoneum. However, it has been suggested that surgery may reduce the risk of both ovarian and breast cancer considerably (about 50%) (Rebbeck, 2000). Reduction in the risk of ovarian cancer has been observed with oral contraceptives (Narod et al., 1998), but it is also suggested that these may increase the risk of breast cancer among BRCA1 or BRCA2 carriers (Ursin et al., 1997; Grabrick et al., 2000).

Cancers other than breast and ovary

A few studies have discovered increased risks of various other cancers among BRCA1 and BRCA2 families, e.g., prostate cancer (Ford et al., 1994; Struewing et al., 1997; Breast Cancer Linkage Consortium, 1999). In our study, as well, an elevated risk of prostate cancer was seen in BRCA2 families, and it was high especially in the age-group 45 to 59. We noticed no increased risk of prostate cancer among BRCA1 families. No other cancers were clustered in our mutation-positive or -negative families. Whether or not to screen for prostate cancer among BRCA1 or BRCA2 carriers remains to be resolved; however, according to this and previous studies (Struewing et al., 1997; Thorlacius et al., 1997; Breast Cancer Linkage Consortium, 1999; Thompson et al., 2001) screening could be reasonable among BRCA2 carriers. Recently, in the BCLC study (Thompson et al., 2001), the risk of prostate cancer by the age of 80 was found to be 33.6% for non-OCCR mutations in BRCA2 and 19.2% for OCCR mutations. Although the value of prostate-specific antigen (PSA) screening has been arguable for the general population, it may be effective for this kind of high-risk population. Matikainen et al. (1999) suggested that serum PSA screening would be a simple and useful measurement in detecting even subclinical cancers in genetically predisposed individuals. Follow-up of any other cancers seems not to be warranted by our results.

Survival of breast cancer patients

Several survival studies have been made among familial or hereditary breast cancer cases. The group is, however, heterogeneous and results inconsistent. In this study, we could categorise the breast cancer patients into BRCA1, BRCA2, and non-BRCA1/2 families and assess their survival. Patients in BRCA1 families tended to have a worse outcome than patients in the sporadic control group. Although the difference was not statistically significant, it is supported by earlier studies indicating that BRCA1 cases have either a similar or a worse prognosis than do sporadic cases (Foulkes et al., 1997; Ansquer et al., 1998; Johannsson et al., 1998; Robson et al., 1999; Foulkes et al., 2000; Stoppa-Lyonnet et al., 2000). However, the prognosis of the four patients in the BRCA1 group with medullary tumours was good: none of them died within the first follow-up years (from 9 to 15 years).

The BRCA2 cases were younger at diagnosis than the sporadic ones, but the overall and age-group-specific survival rates were similar. However, though the BRCA2-associated cancers were more advanced, after adjustment for stage, their relative excess risk of death (0.78, 95% CI 0.39-1.57) appeared to be smaller than that of the sporadic patients. The same tendency of more advanced tumours was seen in the study of Loman et al. (2000). In their study, contrary to ours, relative risk remained elevated even after adjustment for stage (relative risk = 1.6, 95% CI 0.85-3.1).

The survival rate for familial non-BRCA1/2 was better than that of the sporadic patients. This difference was attributable to more favourable stage distribution, and disappeared when adjusted for confounding factors. The survival experience of such a patient group has not been studied earlier.

An advantage of this study was the population-based comparison group comprising all other breast cancer patients diagnosed in Finland during the study period. However, some patients with unrecognised BRCA1 or BRCA2 mutations were obviously among this group. In a study of BRCA1/2 mutations in 1 035 unselected Finnish breast cancer patients (Syrjäkoski et al., 2000), about 2% of all breast cancer patients and 10% of patients younger than 40 years were carriers of BRCA1 or BRCA2 mutations. However,

the dilution of the true contrast due to some BRCA1/2 cases in the control group was very small.

To avoid the selection bias arising because the mutations of living patients are easier to detect than those of dead patients, we included all patients in the families without restriction to confirmed carriers. The potential inclusion of non-carrier patients in the BRCA1 and BRCA2 families may have diluted the differences between mutation carriers and sporadic patients.

Awareness of a family history of cancer may produce a bias resulting from more active self-examination of the breasts or spontaneous mammograms, resulting in breast cancers being diagnosed earlier and at lower stages. However, during the study period (1953 to 1995), neither family cancer screening programs nor mutation analyses were yet available, and there was not much general awareness of hereditary susceptibility to cancer.

We used relative instead of observed survival rates to avoid confounding by the competing causes of death. To make the interpretation of the results easier, we excluded all patients with previous malignancies, because mutation carriers may have a higher risk of multiple cancers than do the sporadic ones, which affects their survival. However, patients with subsequent cancers were not excluded. BRCA1 and BRCA2 carriers have a high risk of ovarian cancer, which may affect the survival of BRCA1- or BRCA2-positive breast cancer patients. In this study, only two subsequent ovarian cancers appeared among patients in the BRCA1 families, and neither of these patients died of ovarian cancer. Thus, the subsequent ovarian cancers did not explain the poorer survival of breast cancer patients among BRCA1 families. Bilateral breast cancer is reported to occur more frequently among BRCA1- and BRCA2-associated patients than among general patient populations (Ford et al., 1994; Breast Cancer Linkage Consortium, 1999). Proper analyses of the effect of bilaterality on survival have not been performed. In our study, the highest proportion of bilateral cases was among BRCA2 families (18.6%); despite this, BRCA2 family patients had a tendency toward better survival. The proportions of bilateral cases between BRCA1 (6.3%) and non-BRCA1/2 (5.7%) families were similar.

For patients with certain hereditary cancers (i.e., hereditary non-polyposis colorectal cancer) better survival rates have been documented than for patients with similar sporadic

cancers (Lynch et al., 1981; Sankila et al., 1996). However, the findings concerning hereditary breast cancer cases have been conflicting, and all studies, including this one, are based on small numbers of cases. Other reasons for conflicting results may be differences in the definitions of a positive family history, in mutation spectrum, in ethnic background, and in disparate control groups.

Among BRCA1- and BRCA2-associated tumours, the tumorigenic pathway and the pathology are different from those of sporadic. Thus, survival may be different as well. The survival rates of breast cancer patients in BRCA1 and BRCA2 families in this study differed, although nonsignificantly, from those of the general sporadic patients. If these small but clinically relevant differences are confirmed in larger study populations, they could have important implications in genetic counselling, in screening, and in future prospects of targeted management of the disease. However, very large studies or meta-analyses are needed.

Future prospects

The last decade has brought many important advances in the understanding of genetic susceptibility to breast cancer. Two major predisposing genes have been found, and we are well in to the understanding of their functional and clinical significance. In addition to clearly pathological protein-truncating mutations, a proportion of BRCA1 and BRCA2 mutations are missense mutations, which change only one amino acid but do not truncate the protein. The relevance of these for possible elevated cancer risk and understanding of the functional significance of the different parts of the genes remains still to be resolved.

Similarly, breast cancer risks associated with mutations in the susceptibility genes may be affected by other modifying factors (genetic, hormonal, environmental), and large genetic-epidemiological studies are needed to resolve the compound effect of such interactions.

Furthermore, it has become increasingly clear that the genetic background of breast cancer is highly heterogeneous. There may be many other genes, dominant or recessive, remaining to be found. Lower penetrance genes or other genetic variants may explain a large proportion of breast cancers. Recently developed technology that could be used in

gene discovery, like cDNA microarrays and serial analysis of gene expression (SAGE) as well as the completion of the Human Genome Project will facilitate an efficient search for new genes (Collins and McKusick, 2001; Polyak and Riggins, 2001).

Identification of novel susceptibility genes and understanding of the different pathways of tumorigenesis of breast cancer will eventually help us to develop better diagnostic strategies, targeted chemopreventive agents, and targeted management of specific subgroups of breast cancer patients and families. Specific gene therapy may become available. In the near future, more information about the magnitude of risk reduction, mortality reduction, and the proper timing of surveillance or prophylactic measures among BRCA1 and BRCA2 carriers is urgently needed.

Conclusions

1. A family history of breast cancer is relatively common among breast cancer patients, as about 30% of the patients reported at least one affected first- or second- degree relative.

2. About 7% of breast cancer patients have such a family history that their families benefit from genetic counselling and may need genetic testing and surveillance.

3. The number of healthy relatives at highest risk who could especially benefit from the recommended presymptomatic screening or preventive measures is not high compared with those in the population screening programmes for specific age-groups already carried out by public health care systems. Based on this study and statistics in 1997, 28 new healthy high-risk women aged 35 to 50 would have been identified in 1997 in Helsinki. If systematic family history screening commenced, the number of identified women would be cumulative in the first years thereafter; however, this is not high compared for example to 19 800 women aged 50 to 58 who were invited to mammography screening in 1997 in Helsinki.

4. Patients know their family history of breast cancer well for first-degree and second-degree relatives. However, breast cancer in more remote relatives is not as well

known. Information on distant relatives may also be important in risk estimations when deciding on screening and surveillance. Patients should be encouraged to discuss cancer in the family.

5. Females in BRCA1- and BRCA2-associated families have a high risk of breast and ovarian cancers. Thus, for them surveillance of both breast and ovarian cancers is needed. Breast cancer patients also have a high risk of subsequent cancers of the breast and ovary, which should be noted in treatment and follow-up decisions.

6. There are families without BRCA1 or BRCA2 mutations that have an increased risk of breast cancer, and breast cancer surveillance is needed also in those families. However, the risk of ovarian cancer was not elevated, and in general, surveillance of the ovaries is not warranted in such families. The high risk of subsequent breast cancer should be noted, as well.

7. Incidence of cancers other than breast and ovary appeared not to be sufficiently increased to warrant special follow-up programmes, except for males among BRCA2 families, who may have an increased risk of prostate cancer; surveillance could be considered by PSA detection.

8. We found no significant difference in the survival of breast cancer patients between BRCA1 or BRCA2 or non-BRCA1/2 families and the general population. Based on this and previous studies it is probable that no major prognostic differences exist.

9. A significant number of our families without BRCA1 or BRCA2 mutations have a high risk of breast cancer, suggesting the importance of one or more yet unidentified susceptibility genes in Finnish families.

Appendix 1.

Health Care Region of Helsinki and Uusimaa

Questionnaire about cancers in the family

Name_____ Identity number_____
Place of birth _____

Age at diagnosis of breast cancer_____
Do you have any other cancers (which?)_____
Please mark on the following lines the site and age at diagnosis if your relative has been affected with cancer. You may use the other side of this paper if needed.

		Cancer	Time of birth	Place of birth
Grandparents:	Maternal grandmother			
	Maternal grandfather			
	Paternal grandmother			
	Paternal grandfather			
Parents:	Mother			
	Father			

Mother's siblings: Number of sisters_____, brothers _____
1_____
2_____
3_____

Father's siblings: Number of sisters_____, brothers _____
1_____
2_____
3_____

Your own siblings: Number of sisters_____, brothers _____
1_____
2_____
3_____

Your own children: 1_____
2_____

Other relatives:_____

_____

_____

I do not wish to be contacted later_____
You may contact me later_____

Thank you for your answers.

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